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Review

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Prostate cancer cells at a therapeutic gunpoint of the autophagy process

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Abstract

In a normal prostate, the process of controling cell death is essential to maintain tissue homeostasis and its inhibition may lead to the development of cancer. Androgen receptor signaling plays pivotal roles in the prostate development and homeostasis as well as in the progression of prostate cancer. The main treatment for prostate cancer is a combination of androgen deprivation therapy (ADT) using anti-androgens and docetaxil administration. However, ADT eventually fails due to a pathological unbalance of cell death processes, in particular apoptosis and autophagy. As a result prostate tumors may re-grow and progress into the castration resistant stage. The role of autophagy in tumorigenesis is complex and it could be a double-edged sword process, as autophagy defects promote cancer progression in association with various dangerous cellular processes, while functional autophagy enables cancer cell survival under stress and likely contributes to the resistance of treatment. Autophagy is often impaired in prostate cancer, due to either activation of the Akt/mTOR pathway, which normally inhibits autophagy, or through allelic loss of Beclin-1 (*BECN1*), an essential autophagy gene. In particular, elucidating the interplay between autophagy and tumor cell metabolism will provide unique opportunities to identify new therapeutic targets and to develop synthetically lethal treatment strategies that preferentially target cancer cells, while sparing normal tissues.

Keywords: Prostate cancer, autophagy, androgen deprivation therapy, mTOR, autophagosome, LC3-II, Beclin-1

PROSTATE CANCER INCIDENCE AND GENETICS

Prostate cancer is a tumor that develops in the prostate, a gland in the male reproductive system. Most prostate cancers are slow growing but there are cases of aggressive forms. Tumor cells may metastasize from the prostate to other parts of the body, particularly the bones and lymph nodes. Prostate cancer may cause pain, difficulty in urinating, problems during sexual intercourse, or erectile dysfunction. In particular,

Table 1. Main genes involved in prostate cancer

Gene	Full name	Function/references
BRCA1	Breast cancer susceptibility protein type 1	DNA repair[7]
BRCA2	Breast cancer susceptibility protein type 2	DNA repair[7]
HPC1	Hereditary prostate cancer	Prostate cancer susceptibility ribonuclease L[8]
VDR	Vitamin D receptor	Inhibition of cell growth, metastasis and angiogenesis; apoptosis modulation and cell differentiation[8]
CD82	Cluster of differentiation 82	Metastasis suppressor attenuates the matrix adhesion[9]
PTEN	Phosphatase and tensin homolog	Tumor suppressor and cell cycle regulation[9]
mTOR	Mammalian target of rapamycin	Key signaling pathway linked to tumorigenesis and resistance to therapy[10]
PSA	Prostate specific antigen	Dissolver of cervical mucus, allowing the entry of sperm in the uterus[11]
BCL2	B-cell lymphoma 2	Pro-survival protein associated with the development of androgen-independent prostate cancer[12]
MKI67	Antigen Ki-67	Nuclear protein involved cell proliferation[13]
ERK-5	Mitogen-actvated protein kinase 7	Signaling processes of various receptor molecules. In response to extracellular signals, this kinase translocate to the nucleus, where it regulates gene expression and activates different transcription factors[14]
SP1	Transcription factor Sp1	Involved in many cellular processes, including cell differentiation, growth, apoptosis, immune responses, DNA damage, and chromatin remodeling[15]
TPD52	Tumor protein 52	Unknown[16]

prostate cancer tends to develop in men over the age of fifty[1]. Rates of detection of prostate cancers vary widely across the world, with South and East Asia detecting less frequently than in Europe and in the United States. Globally, it is the sixth leading cause of cancer-related death in men[2]. More than 200,000 new cases are estimated in the United States in 2013, with a mortality rates over per 10 cases. Moreover, there are different ways of classifying patients with prostate cancer: the tumor-node-metastases (TNM) classification of malignant tumors evaluates the extension of the tumor, the involvement of lymph nodes and the metastatic dissemination. The Gleason Grading system is additionally used to evaluate the prognosis of men with prostate cancer. A Gleason score is given to prostate cancer based upon its microscopic appearance: cancers with a higher Gleason score are more aggressive and have a worse prognosis[3,4].

Many factors, including genetics and diet, have been implicated in the development of this cancer. As suggested by association studies, genetic background can contribute to prostate cancer risk with family, race, and specific gene variants. Men who have a first-degree relative (brother or father) with prostate cancer have twice the risk of developing the cancer, and those with two first-degree relatives affected have a fivefold greater risk compared with men with no family history[5]. Studies of twins in Scandinavia suggest that 40% of prostate cancer risk can be explained by inherited factors[6].

A summary of the different genes implicated in prostate cancer are highlighted in Table 1[7-16].

METABOLISM AS A PRIVILEGED TARGET IN PROSTATE CANCER CELLS

Different metabolic targets and sub-targets in prostate cancer

The specific alterations in metabolic pathways observed in cancer cells confirm that tumors need unusual amounts of energy and biosynthetic precursors to survive and grow[17].

However, the unique intermediary metabolism in prostate cancer cells is substantially different from that found in other cancer cell types[18]. In particular to satisfy the energy demand and to generate ATP, most cancer cells are mainly derive energy from aerobic glycolysis[19]. In androgen-dependent prostate cancer cells, Warburg[19] has demonstrated that glucose does not play a major metabolic role because LNCaP cells, and androgen-sensitive human prostate adenocarcinoma cells[20] widely employed in *in vitro* prostate cancer studies, can even grow in presence of low glucose concentrations[21]. Therefore, the metabolic state of prostate cancer cells is altered to satisfy the increased demand for energy that is required to support the

stimulated protein synthesis; moreover, these cells also have an inefficient autophagy process due to reduced catabolism[22]. The inhibition of glycolysis by the promoting some kind of metabolic stresses may be used to improve therapies. A novel therapeutic paradigm was the treatment introduced by DiPaola *et al.*[23], using 2-Deoxy-D-glucose (2DG), an inhibitor of glycolysis and a glucose analogue that blocks the uptake of glucose and induced cytotoxicity and autophagy in different prostate cancer cells. It was, therefore, hypothesized that prostate cancer is metabolically fragile because of dependence on glycolysis and impaired autophagy.

Interestingly, altered lipid metabolism has also been demonstrated by multiple groups to play an important role in prostate cancer progression[24]. Fatty acid synthase (FAS), a rate-limiting enzyme in *de novo* lipogenesis, is frequently over-expressed in prostate cancers[25,26]. Correspondingly, pharmacological or molecular inhibition of either FAS or other lipogenic enzymes, like acetyl-coenzyme A carboxylase (ACC) and ATP citrate lyase (ACL), suppressed both *in vitro* and *in vivo* tumor growth[27,28]. FASs are also stimulated by androgen hormones as seen in LNCaP cell accumulation of lipid droplets (LDs) within the cytoplasm[29] containing both triacylglycerols (TGs) and cholesterols, which are enveloped by a monolayer of phospholipids and associated proteins[30]. LDs can be metabolized by hormone-regulated cytosolic lipases that break down the TGs into fatty acids which are then utilized for β-oxidation[31], but there is a second pathway involving lipolysis mediated by autophagy. Recently, Singh *et al.*[32] reported that in rat hepatocytes, autophagosomes sequestered LDs and caused lysosomal lipolysis. An alternative pathway of lipolysis has been observed also in prostate cancer cells, further explaining how prostate cancer cells may adapt to survive in hostile environmental conditions[33]. Although androgens promote prostate cancer cell growth in part by increasing the expression of several of these lipogenic enzymes[24,34,35], it is not known whether androgens may promote the formation of these lipid reservoirs by additional mechanisms that may also be critical for tumorigenesis[36].

Statins (or HMG-CoA reductase inhibitors) are a class of drugs used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver. Statins such as atorvastatin (ATO), in addition to their effects on cholesterol biosynthesis, have attracted considerable interest for their possible utility in cancer prevention and therapy[37]. It has been demonstrated by *in vitro* studies that autophagy and autophagy-associated cell death in PC3 prostate cancer cells can be induced by ATO[38]. Clinically, lowering of serum cholesterol is the first effect of statin treatment; even though the inhibition of prostate cancer cell growth could seem independent from the lowering of serum cholesterol, both can be mediated by effects on the mevalonate pathway. Recently, it has been discovered that ATO inhibited the synthesis of geranylgeranyl pyrophosphate (i.e. an intermediate in the HMG-CoA reductase pathway used by organisms in the biosynthesis of terpenes and terpenoid), played an important role in the induction of autophagy and suppressing prostate cancer cell growth[39]. Specifically, the authors found a stress-responsive miRNA, called miR-182, which mediates the activity of ATO in prostate cancer cells.

A landscape of ADT in prostate cancer

In patients with advanced prostate cancer, ADT remains the most effective standard treatment, inducing programmed cell death and inhibiting cell proliferation[40]. Unfortunately, after short term remissions, cancer cells may escape from this treatment, survive and develop androgen-independent growing capabilities by several mechanisms[41]. Surviving tumor cells shows a phenotype known as Castration-resistant prostate cancer (CRPC) and death usually occurs within 3 years in the majority of patients[42]. The principal androgens produced in the testes are testosterone and the more active metabolite dihydrotestosterone. Androgens work after binding and trans-activating androgen receptor (AR), which regulates gene expression by interacting with different co-regulators during prostate cancer progression. The down-regulation of the levels of androgens, or preventing their entrance into prostate cancer cells, can reduce the tumor growth. To date, there are many hormone therapy protocols to achieve this goal. ADT is now performed with surgical castration (bilateral orchiectomy) or with luteinizing hormone-releasing hormone (LHRH) agonist therapy.

LHRH can improve a disease-free phase and a moderate survival (if it's combined with primary radiation), reducing circulating testosterone levels to so-called castrate levels (< 0.5 ng/mL). Anti-androgen therapy is part of the common hormone therapy that is used with drugs which can stop the action of particular hormones. Presently, the anti-androgen therapy is always combined with orchiectomy or with LHRH agonists as a first-line hormone therapy, referred as combined androgen blockade (CAB). During the first days of treatment with LHRH analogues there could be an overload of testosterone: to counteract this event, specific LHRH's antagonists have been proposed[43].

At the first symptoms of metastasis, in CRPC patients, the cytotoxic chemotherapy is usually initiated[44]. Although cancer cells still express ARs, at some point they no longer respond to ADT, and prostate cancer become recurrent[45]. It has been discovered that there are some *AR* mutations often expressed in hormone-refractory prostate cancer and these mutations cause a deregulation of transcriptional activity. These events are in contrast with the purpose of targeted therapies designed specifically to inhibit the receptor functions[46]. Eventually, it has been studied that prostate cancer cells can resist to ADT, surviving and developing an androgen independence in different ways, such as stimulating growth factor pathways, activating stress-dependent survival genes, increasing cytoprotective chaperone networks, and escaping from apoptosis processes[47-49].

The regulatory effects of androgens on prostate cancer cells are still debated; in particular, the effects of modulation of the autophagy process during androgen deprivation have been investigated[50]. Previously, it was observed that autophagy was induced if androgen-sensitive LNCaP cells were cultured in the absence of serum; otherwise, if dihydrotestosterone was introduced, the autophagic process was reduced. This suggests that specific androgenic hormones produce a down regulation of autophagy process[51]. In addition, two independent studies have shown that cell death increases if LNCaP cells undergo androgen deprivation, suggesting that autophagic might exert a protection role toward prostate cancer cells[51,52].

THE DIFFERENT EFFECTS OF AUTOPHAGY MODULATION IN PRECLINICAL MODELS OF PROSTATE CANCER CELLS

The autophagy process

Autophagy is a homeostatic process whereby cellular components are engulfed into vesicles known as autophagosomes, which then fuse with lysosomes and are consequently subjected to proteolytic degradation[53].

In 1963 the Nobel Laurate, Christian de Duve, introduced the concept of autophagy[54], now this definition has been assigned to several intracellular processes, including micro- and macro-autophagy, chaperone-mediated autophagy, and all of them eventually converge towards a common degradation phase mediated by lysosomes[55,56].

Macro-autophagy, generally referred to as autophagy, has been experimentally proven to be involved in the pathogenesis of different diseases including cancer[57].

At a molecular level, the kinase mTOR is a critical regulator of autophagy induction, with activated mTOR (MAPK and Akt signaling) it suppresses autophagy, whereas a negative regulation of mTOR, p53 and AMP-activated protein kinase (AMPK) signaling, promotes it. Three related serine/threonine kinases, UNC-51-like kinase -1, -2, and -3 (i.e. ULK1, ULK2 and UKL3) act downstream of the mTOR complex. ULK1 and ULK2 form a large complex with the mammalian homolog of an autophagy related gene product (mAtg13) and the scaffold protein FIP200. Class III phosphoinositide 3 kinase (PI3K) complex, containing hVps34, Beclin-1, p150, and Atg14-like protein or ultraviolet irradiation resistance-associated gene product (UVRAG), is required for the induction of autophagy. Autophagosome formation in controlled by Atg genes proteins through Atg12-Atg5 and LC3-II complexes. Atg12 is conjugated to Atg5 in an ubiquitin-like

reaction that requires Atg7 and Atg10. The Atg12-Atg5 complex then interacts non-covalently with Atg16 to form a larger complex. LC3/Atg8 is cleaved at its C-terminus by Atg4 protease to generate the cytosolic LC3-I. LC3-I is then conjugated to phosphatidylethanolamine following an ubiquitin like reaction that requires Atg7 and Atg3. Then, the lipidated form of LC3, known as LC3-II, is attached to the autophagosome membrane.

An extensive crosstalk and a dynamic balance exists between apoptosis and autophagy. Autophagy is a survival mechanism that typically is switched on during a nutrient deficiency; however, its excessive activation can lead to cell death, with morphological features different from apoptosis ones. Proteins typically placed at the cross roads of this processes are Beclin-1 and Bcl-2. In particular, Beclin-1-dependent autophagy is inhibited by Bcl-2, which works as an anti-autophagic regulator and as a pro-survival mechanism[58]. Autophagy, like other metabolic pathways, can be regulated by various inducers and inhibitors. For example, autophagy can be induced by deprivation of amino acids or serum, whereas it can be reduced by 3-methyladenine (3-MA), an inhibitor of class III PI3K, that blocks the generation of phosphatidylinositol 3-phosphate (PI3P), an essential docking molecule for the formation of phagophores at early stage of autophagy. In addition, to investigate the autophagic flux some antibiotics are used such as bafilomycin A1 and concanamycin A, because they inhibit specific ATPase activities and acidification of the lysosome, and therefore the final fusion event between the lysosomes and autophagic vesicles[58].

The process of autophagy has been identified as an important mechanism of cellular resistance, or alternatively of cell death[59,60]. Autophagy is a response to the cell's energy demand, whereby the loose cytoplasm and the cellular organelles undergo lysosomal degradation to compensate for the demand for alternative energy during periods of nutritional limitation. Besides the recycling of nutrients, autophagy also plays a role for degradating damaged organelles by proteolysis to maintain a cellular quality control.

A combined inhibition of autophagy and proteasome degradation pathway induces an accumulation of intracellular protein aggregates reminiscent of neuronal inclusion bodies, causing a significant cancer cell death than blocking the proteasome degradation pathway alone. As a result, proteasome inhibition activates autophagy via a eukaryotic initiation 2 alpha-dependent mechanism to eliminate protein aggregates and alleviate proteotoxic stress[61]. On the other hand, sustained autophagy under conditions of protracted starvation has also been proposed to lead to cell death; thus, the survival or death consequences of autophagy are condition-dependent[62-65]. Therefore, in cancer, autophagy has a controversial role, it can protect cancer cells from adverse conditions or induce the death of cancer cells. In particular, in human prostate cancer, autophagy is often impaired due to allelic loss of Beclin-1 locus[66] or to the activation of the PI3K kinase/Akt/mTOR pathway that finally inhibits autophagy. It has been demonstrated, in particular in *in vitro* studies on epithelial prostate cancer cells, that autophagy can provide a survival mechanism for cells that are undergoing some kind of starvation, favoring tumor growth[67].

Autophagy in prostate cancer

As molecular events, cancer development is often associated with deletion or silencing of tumor suppressors genes such as PTEN, a negative regulator of the PI3K/Akt/mTOR pathway[68], leading to resistance to various therapies in both preclinical and clinical trials[69]. Therefore, the PI3K/Akt/mTOR pathway plays a central role in various cellular processes, including protein cell survival, motility, synthesis, cell cycle, cell growth, and angiogenesis. The deregulation on this pathway may contribute to the malignant phenotype. Many small-molecule inhibitors targeting Akt, mTOR, and/or PI3K, and typically promotes growth arrest rather than cell death in solid tumors, and, therefore, use of small molecule inhibitors have been limited[70]. However, some of them have been successfully used in prostate cancer therapeutic schemes. In particular, some prostate cancer cell lines such as PC346-Flu1 and LNCaP were sensitive to monotherapy with the novel AKT inhibitor AZD5363, resulting in an increase in apoptosis at concentrations achievable in preclinical

models[71]; on the contrary, other prostate cancer cell lines, such as PC3 and DU-145, were quite resistant to the treatment. Recently, Lamoureux *et al.*[72] showed that AZD5363 induced cell death in the drug-resistant prostate cell lines by means of a chloroquine-mediated autophagy inactivation.

Chloroquine is known as a drug for the treatment of rheumatoid arthritis and malaria and to achieve an anti-HIV activity[73]. Chloroquine may be a clinically effective drug in prostate cancer, due to its ability to block lysosome acidification, preventing fusion with autophagosomes and, therefore, inhibiting the autophagy process[74]. Currently, many clinical trials used chloroquine to increase the effects of different targeted therapies such as bortezomib, temsirolimus, or gemcitabine in various cancers[75]. Early antitumor activities have been demonstrated in some of these trials. Furthermore, some studies evidenced that breast cancer cells could be sensitized to cisplatin by chloroquine, in an autophagy inhibition-independent manner, irrespective to Atg12 or Beclin-1 expression[76]. Previous studies reported that cell death in breast cancer[77] and in glioma cells[78] is increased by the combination of chloroquine (or other lysosomotropic agents) and PI3K pathway inhibitors. It was demonstrated that *in vitro* administration of D,L-sulforaphane (SFN), a synthetic racemic analogue of broccoli constituent L-sulforaphane, can inactivate histone deacetylase 6, therefore, interfering with the expression of androgen receptor genes in prostate cancer cells[79]. However, SNF also induced a cytoprotective autophagy in cultured human prostate cancer cells and it can be further enhanced with the pharmacologic inhibition of autophagy using chloroquine. The combined treatment was associated with decreased cell proliferation, increased apoptosis, alterations in protein levels of autophagy regulators Atg5 and phospho-mTOR, and suppression of biochemical features of epithelial-mesenchymal transition[80].

Pyruvate kinase isoenzyme type M2 (PKM2), a modulator of glycolysis, also regulates the autophagy process by up-regulating LC3B or Beclin-1 in glioma cells or in cancer-associated fibroblasts[81,82]. Since Sp-1 directly regulates PKM2, Ling *et al.*[15] (2017) have recently found that a specific microRNA, miR-361-5p, inhibits CRPC cell proliferation, metabolism, and autophagy by directly targeting Sp1/PKM2 signaling, which is a potential target in PCa therapy.

Recently, it has been reported that the retinoic acid receptor responder (RARRES1)/tazarotene-induced gene-1 (TIG-1), a novel retinoid inducible gene first identified in skin raft cultures, modulates a series of signaling pathways inducing autophagy and inhibiting angiogenesis. The over-expression of *RARRES1* can lead to the block of MAPK, to the increase of Beclin-1, Atg3, LC3-II protein expression, and finally, the inhibition of mTOR expression[83]. These studies strongly indicated the attractive prospect of blocking autophagy processes combined with targeted therapy as a promising therapeutic approach for prostate cancer[72].

Zeng *et al.*[84] (2018) have very recently investigated the role of a prostate leucine zipper protein (PrLZ) in combination with docetaxel-(the first-line standard approach in PCa) resistance in PCa, focusing on PrLZ-regulating autophagy pathway. PCa cells are protected from docetaxel induced-apoptosis by overexpression of PrLZ. The negative regulation of autophagy by PrLZ is mediated through LKB1/AMPK signaling pathway. The autophagy pathway and PrLZ can become a good therapeutic target for CRPC and, especially, docetaxel-resistant CRPC therapy[84]. Autophagy has, generally, a protective function on cancer cells so maybe, if autophagy is properly inhibited, it could be a clinical strategy to contrast therapeutic resistance in prostate cancer, when is associated with partial failure of radiation or chemotherapeutic schemes[85]. On the contrary, in androgen-independent prostate cancer cells, it has been shown that autophagy induction may sensitize cells to radiation[86]. Despite these contrasting results, a therapeutic benefit for prostate cancer patients can come from either induction or inhibition of autophagy, depending on the specific tumor environment, and ultimately, to the adopted therapeutic scheme[49]. Radiation therapy is a cytoprotective autophagy inducer in prostate cancer cells[87], it was also reported that incubation of LNCaP cells in serum-free medium lead to a pro-death autophagy process[67]. Li *et al.*[51] found out that the inhibition of autophagy can lead LNCaP

cells to apoptosis in a serum-free medium, but not in cells in medium with serum or dihydrotestosterone. This suggest that autophagy process during androgen deprivation can protect LNCap cells from death. In other cancer cell lines it has been demonstrated that autophagy is modulated by growth factors contained in serum through activating the mTOR pathway[75].

Cell death, in certain androgen deprivation situations under *in vitro* condition on epithelial prostate cancer cells, can arise by blocking autophagy processes via interfering with genetic or pharmacology means.

Furthermore, it has been observed that there is a parallelism between autophagy stimulation by androgen-ablation in prostate cancer cells and autophagy induction in some breast cancer cells during anti-hormone therapy. It was hypothesized that breast cancer cells tend to increase autophagy levels to develop a resistance to anti-estrogens[88]. To this regard, chloroquine induce cell death in LNCaP cells in a time and dose-dependent way, combined with an androgen deprivation[89]. At the same time, efficacy of androgen-ablation cell death can be enhanced by a combination of pharmacological inhibition of autophagy and chemotherapy[90]. Additional drugs that potentially are known to interfere with autophagy flux include bafilomycin A1, 3-methyladenine and pepstatin A[91]. However, these pharmacological molecules produces many off-target effects in different cellular pathways[91].

At this moment there are not enough *in vivo* studies on the combination of androgen deprivation and autophagy inhibition, but the *in vitro* results obtained to date show the potentiality of the combination of conventional ADT and autophagy-modulation in prostate cancer patients[50].

Autophagy and androgen receptor interplay

In regulation of prostate development as well as in carcinogenesis, AR is a critical transcription factor, but in the autophagy process, the role of AR remains poorly understood[92]. In fact, in PC3 AR-negative cells, statin is an autophagy inducer, but not in LNCaP AR-positive cells[93]. In contrast, in LNCaP cells autophagy process is inhibited by dihydrotestosterone treatment, but this does not happen in PC3 cells[51]. In addition, other studies showed that cell death may increase, under androgen deprivation by inhibiting autophagy process in LNCaP cells and suggesting a role of autophagy as a protector of prostate cancer cells[93,94]. Due to these contrasting results, in prostate cancer cell, the role of androgen/AR signals in altering autophagy remains unclear[95]. Traditional androgen deprivation therapy to treat prostate cancer may not reverse the AR regulated autophagy pathway because this pathway was found under different conditions at different androgen concentrations. In particular, Jiang *et al.*[95] have used the compound ASC-J9 to specifically degrade AR in AR-positive cells.

Results revealed increased autophagy and decreased cell growth compared to those of sham-treated AR-positive cells. Therefore, targeting AR to promote autophagy may represent a new potential therapeutic approach to prostate cancer[39].

It is emerging that different mechanisms regulate the autophagy process in androgen-ablation conditions[96]. In case of hypoxic conditions, autophagy can be induced by different independent pathways including the inhibition of mTOR kinase and hypoxia-inducible factors (HIF-1). Another mechanism that activate an autophagic response is controlled by energetic stress[94]. In particular, androgen deprivation may cause the genesis of autophagic vesicles which incorporate LD. The catabolism of lipids, known as lipophagy, represents a way to support energy demand and helps in the surviving of cells during ADT[25]. The loss of energy production leads to an activation of AMPK which, again, leads to suppression of mTOR signaling; this events cause fatty acid oxidation, glycolysis[97] and, lastly, autophagy[98]. It is very interesting that about 40% human prostate cancers have an over-expression of AMPK, which confirms its activation in different metabolic pathways[99].

A recent work by Scherz-Shouval *et al.*[100] showed that elevated levels of reacting oxygen species (ROS) activate autophagy. In addition, it was highlighted that androgen-mediated ROS generation promoted prostate cancer cell growth[101], which provided the rationale that androgenic regulation of autophagy required a specific ROS signal. This evidence was recently further confirmed, reporting that elevated ROS levels contributed to the androgen-induced autophagy, to intracellular lipid accumulation, and finally to tumor cell growth[36]. Overall, it is clear that the regulation of ROS levels within the cells is critical: although too much ROS can trigger apoptosis, moderate levels promote cell signaling activities that are needed for both proliferation and survival[102].

Autophagy and apoptosis crosstalk in prostate cancer

It is known that autophagy is particularly important as a survival mechanism in tumors with defects in the apoptotic pathway, supporting an already suggested therapeutic paradigm of a dual apoptotic and autophagic inhibition[103]. Prostate cancer cells could be sensitized to different apoptotic stimuli by inhibiting autophagy, which happens during ADT. In fact, appropriate stimuli can lead prostate cancer cells to apoptosis, even though these cells tend to evolve into an androgen-resistant phenotype[104]. To this regard, a recent investigation by Saleem *et al.*[105] demonstrated that employing the well-established Bcl-2 inhibitor, ABT-737, in combination with chloroquine resulted in enhanced cytotoxicity in prostate cancer *in vitro* and *in vivo*. These results also highlighted the importance in clinical studies for the evaluation of the crosstalk pathways between apoptosis and autophagy[100]. Tumor necrosis factor-alpha (TNF-α) and TNF-related apoptosis-inducing ligand (TRAIL), members of the death receptor ligand superfamily, as apoptotic markers, have been suggested as potential anti-prostate cancer pharmacological targets[106,107]. In the LNCap cells the apoptotic response was enhanced by inhibiting pharmacological autophagy. Furthermore, the apoptotic cytotoxicity induced by TRAIL, in prostate cancer cell lines, was effectively increased by blocking autophagy by siRNAs targeting autophagic genes such as *BECN1* or *ATG7*[108].

Shin *et al.*[109] reported that docosahexaenoic acid (DHA), an omega-3 fatty acid present in cold-water fatty fishes, leads to mitochondrial ROS generation and reduces phospho-mTOR and phospho-Akt expression levels in concentration-dependent manner in p53-mutant DU145 and PC3 cells. These results suggest that DHA may be beneficial for patients with p53-mutant prostate cancer and show its possible use in clinical therapies[109].

Many natural compounds are studied for their antitumor features. Recently, the effects of Marchantin M (Mar), a naturally occurring macrocyclicbisbibenzyls, have been tested, which resulted in a favorable apoptosis modulation[110]. Through this observation, it was hypothesized that caspase-independent mechanisms can also contribute to its cytotoxic effect on prostate cancer cells. Very recently, Jiang *et al.*[111] revealed that the Mar-induced cell death was additionally associated with the activation of autophagy, together with the induction of ER stress and the inhibition of proteasome activity. These results enforced the goal of the identification of chemotherapeutic compounds able to trigger apoptotic as well as autophagic cell death in prostate cancer cells, for a successful application in cancer therapy.

Novel molecular actors for autophagy tuning in prostate cancer models

Recent contributions highlighted tyrosine kinases (TKs) and histone deacetylase (HDAC) inhibitors as promising modulators of autophagy activity in novel therapeutic schemes in prostate cancer models[112]. It was reported that TKs play a key role in tumor sensitivity to radiation and chemical-induced apoptosis[113]. Non-receptor tyrosine kinases (NRTK) are shown to participate in processes such as cell proliferation and migration in prostate cancer. There are several NRTK families, classified based on their structural similarities, that might potentially interfere with cell death balance in prostate cancer[23-25]. In particular, it has been shown that the administration of autophagy interfering molecules or drugs sensitized these cells toward Src tyrosine kinase inhibitor-based therapies[114]. Specifically, AR is phosphorylated by Src kinase complex, resulting in AR nuclear

translocation and activation; it was additionally reported that this kinase played an important role in the development of castration-resistant disease state[115]. Indeed, tyrosine kinase inhibitors targeting Src can inhibit androgen-independent growth of prostate cancer cells, but did not induce significant apoptosis. Therefore, an autophagy blocking strategy might significantly potentiate the effects of tyrosine kinase inhibitors as pro-apoptotic inducers[116]. In addition to cell migration, Src assists in tumor invasion through its regulation of matrix metalloproteinases (MMPs) via degradation of the extracellular matrix. Another interaction that involves Src in CaP is with steroid receptors. It has been demonstrated that in low androgen conditions, AR can activate Scr in the cytoplasm, thereby triggering downstream signaling events independent of AR transcriptional and DNA-binding activity[38,48]. In fact, DNA synthesis can be inhibited by Src (as a dominant negative factor) after stimulation with low amount of androgens, but the Scr pathway can be bypassed with higher concentrations of androgen coupled with AR over-expression. Scr in addition to binding with AR, if stimulated with estradiol, can also interact with the estrogen receptor (ER) and thereby promote cell proliferation[38,49,50]: thus, it can be hypothesized that Src serves as a scaffolding protein for the AR-ER complex.

Focal kinase (FAK) adhesion, in addition to migration and proliferation, may also be involved in angiogenesis and apoptosis in CaP cells. There are evidence that FAK induces vascular endothelial growth factor (*VEGF*) transcription in an ERK1/2-dependent, Rap1-dependent, and Raf-dependent but Ras-independent manner[91-93].

PTEN, a tumor suppressor gene with dual phosphatase activity, is part of the negative FAK regulators, and is deleted in the aggressive CaP[94]. The formation of the Lyn-PI3K-NEP complex can be regulated indirectly, in a negative way, by FAK[60]. ETK/BMX complex, discovered in 1994, belongs to the Tec family of NRTK[117]. In CaP, ETK is downstream of PI3K on the induction of the neuroendocrine differentiation following IL-6 stimulation in LNCaP cells[118]. It is also know that it works as an anti-apoptotic factor. Ove-expression of ETK leads to a resistance to apoptosis in CaP cells due to its interaction with PI3K[118]. The activation of EKT do not require PI3K[27]. Rather, the interaction of ETK with p53 could be another mechanism of protection against apoptosis[119]. The introduction of ETK C-terminal fragment into PC-3 cells lead to apoptosis after proteolytic cleavage of ETK by caspases[120]. ETK is a signal transducer between SRC and AR downstream and FAK upstream. However, ETK alone is not enough efficient to activate AR, since it requires to interacts with Pim1 protein[117,121].

Several studies have suggested that inhibition of HDAC in the progression of autophagy could be a new way for treatments of prostate cancer[122]. It is known that HDAC inhibitors are among the most promising targeted anticancer agents and are potent inducers of growth arrest, differentiation, and autophagic cell death of prostate cells[123]. Very recently, Patra *et al.*[124] developed a novel HDAC inhibitor (MHY219) that induced cancer cell death and might be employed as a chemotherapy adjuvant in clinical studies. Similarly, other HDAC inhibitors have been tested in prostate cancer studies[125-127]. In another recent contribution, Vallo *et al.*[122] assayed PXD101, a potent pan HDAC inhibitor, to prevent the onset of castration-resistant phenotype and to potentiate hormonal therapy. A very interesting aspect is that there is a functional link between HDAC and liver X receptors (LXRs) members of the nuclear receptor family that regulates intracellular lipid homeostasis[128]. As already mentioned, lipids play a complex role in the progression and maintenance of prostate cancer. In fact, the increasing *de novo* synthesis of cholesterol and/or fatty acids is associated with the development of prostate cancer. Therefore, by inhibiting HDAC it was possible to reduce the levels of intracellular cholesterol and consequently it reduced the proliferation of tumor cells. Inhibitors HDCA and LXRs can, therefore, inhibit the proliferation of tumor cells[128].

Currently, the only drug, approved to be applied in the chemotherapy of PCa, is docetaxel. Recently, a new drug was introduced, Salen-MN, a novel type of synthetic reagent bionic and exerts remarkable anticancer activities, but its effect is not been completely elucidated in PCa. In particular, treatment with Salen-Mn inhibited growth in PC-3 and DU145 cells. Moreover, Salen-Mn *in vitro* administration induced

an increase in the expression of apoptotic proteins such as Bcl-2-associated X protein (Bax), cleaved poly (ADP-ribose) polymerase (PARP), and cleaved caspase-3. Furthermore, it has been observed that Salen-Mn induced expression of LC3-I/II in both dose- and time-dependent manner. It was documented that Salen-Mn increased autophagy by means of AMPK phosphorylation. Therefore, Salen-Mn might represent a novel promising candidate for the treatment of prostate cancer[129].

CONCLUDING REMARKS

Basal autophagy helps to maintain homeostasis by contributing to organelle and protein turnover, but it is also a survival mechanism that is efficiently induced in stressed cells. Autophagy defects have been implicated in various health states and diseases, including infection, myopathy, Crohn's disease, neurodegeneration and cancer. However, the role of autophagy in cancer is quite complicated and still somewhat controversial; it appears to be tumor suppressive during cancer development, but contributes to tumor cell survival during cancer progression. Furthermore, tumor cells can use autophagy to resist to various anticancer therapies. Cancer cells experience higher metabolic and energy demands and exposed to stresses than normal cells because of their rapid proliferation and altered glycolytic metabolism. These cells depend more heavily on autophagy for survival.

The therapeutic benefits of various cancer therapies have been improved because of the inhibition of autophagy, which allows a methodology to specifically target cells characterized by higher levels of autophagy. There is still much to be discovered about autophagy and its regulation, but the ongoing results are delineating a promising pharmacological target for cancer treatment. However, it is necessary to discover additional biomarkers to evaluate the complex dynamism of autophagy processes and to establish new methods to assess autophagy in clinical samples.

The data here reviewed from the current scientific literature generally indicated that the modulation of autophagy may be therapeutically beneficial in various tumors because of their ability to sensitize cancer cells to the different therapies, including DNA-damaging agents, anti-hormone therapies and radiation and chemotherapeutic combined strategies.

In particular, it is emerging that in prostate cancer, a promising combined treatment during androgen deprivation therapy is to target metabolic stress-induced signaling pathways. These complex pathways are intimately controlled by various molecular actors that play important roles in programmed cell death pathways including autophagy and apoptosis. In particular, autophagy is clearly becoming a central regulator of the main physiological and pathological processes, which through a precise and sensitive balancing determine pro-death or pro-survival fate of the cell. Therefore, the modulation of autophagy process in malignant cell types can be regarded as a potential strategy in cancer therapy.

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Authors' contributions

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Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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