Review

Combined therapies with taxanes based-chemotherapeutic drugs in prostate cancer: novel insights and future directions

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Abstract: The oncologic disease is a big global health issue that causes thousands of deaths annually, and it has a significant impact in the life quality of patients. Prostate cancer (PCa) is the second most diagnosed cancer and the fourth leading cause of cancer-related death in men in the western world. Delineation of pathogenetic pathways and key driver molecular alterations involved in PCa development has provided a roadmap for the evaluation of biomarkers in predicting disease outcome and to identify potential therapeutic targets. Chemotherapeutic agents introduced from the 1990s include the taxanes (paclitaxel, docetaxel and cabazitaxel), which are the most anticancer drugs used for PCa treatment. This review presents the current knowledge about the onset and development of PCa, state-of-art on the use of taxane-based therapy, and their combination with targeting different transmembrane oncoproteins in PCa. The silencing of some transmembrane proteins can improve taxane sensitivity, and therefore, may be a mechanism to improve the effectiveness of these drugs in PCa treatment. This combined therapy needs to be explored as potential therapeutic agent for reducing cell proliferation, migration, and invasiveness in PCa.

Keywords: PCa; taxanes-based drugs; combination therapy; transmembrane proteins.

1. Introduction

The burden of cancer incidence and mortality is rapidly growing worldwide, and expectations for 2020 pointed to, approximately, 19.3 million new cancer cases and 10.0 million cancer deaths [GLOBOCAN, https://gco.iarc.fr/, accessed on 9th March 2023]. Prostate Cancer (PCa) is currently the second most common cancer in men and represents the fourth leading cause of cancer-related mortality. In 2020, 1.4 million new cases of PCa were diagnosed worldwide and, approximately 375,000 associated deaths were reported by World Health Organization [1]. The increased number of PCa can be explained by the lack of comprehensive national control programs that contributes to substantial disparities in early detection of cancer and management of these patients, with a 3-fold higher incidence rates in countries with high human development when compared to countries with low human development (37.5 and 11.3 per 100,000 habitants, respectively), although mortality rates are less variable (8.1 and 5.9 per 100,000 habitants, respectively) [2, 3]. Moreover, the aetiology of PCa is multifactorial and remain largely unknown, when compared to other types of cancer. Epidemiologic evidence has identified several biological and genetic factors, but also environmental and lifestyle factors have been shown to contribute to the appearance and progression of PCa, namely advanced age, family history and genetic predisposition, ethnicity, smoking and alcohol consumption, obesity and metabolic syndrome, physical inactivity, diet and nutrition, medications, sexual activity and vasectomy, hormones, infection, inflammation, and chemokines [4, 5]. However, age is considered the highest risk factor for the development of PCa. The peak of incidence is found in older men with approximately 70-74 years old [6].

Currently, several agents received FDA approval and have been associated with beneficial effects in improving survival and life quality in patients with this pathology, including abiraterone, enzalutamide, apalutamide, and darolutamide (inhibitors of the androgen axis); paclitaxel, docetaxel and cabazitaxel (target microtubules by inhibiting depolymerization or promoting polymerization); radium-223 (radioactive agents as target bone metastases); and sipuleucel-T (trigger cellular immune mechanisms) [7]. From those agents, an appropriate drug selection is done according to clinical usage for the treatment of PCa. Several cancers are treated with drug combination, but PCa has remained an exception [8]. Transmembrane proteins are involved in many crucial cell processes, including signaling transduction pathways, transport of ions and molecules, protein targeting and intracellular transport, as well as membrane trafficking [9]. Moreover, since membrane proteins are involved in essential cellular pathways, they are often recognized in the pathophysiology of many diseases and are major targets for pharmaceutical agents, with more than 60% of drug targets being transmembrane proteins [10]. Hence, developing the effective combination of drugs and targeting some transmembrane proteins can provide insights concerning new therapeutic strategies for advanced stages of PCa. This review provides an overview of the development of PCa, and it is focused on the taxanes-based therapy currently used. Therefore, it was analyzed the scientific literature concerning the combined action of taxanes based-chemotherapeutic drugs with inhibition of transmembrane oncoproteins within the paradigm of PCa.

2. Onset and development of PCa

The human prostate gland is the major accessory gland of the male reproductive system, located frontal to the rectum and immediately below the urinary bladder, surrounding prostatic urethra and the ejaculatory ducts [11, 12]. Normal prostate tissue consists of prostatic ducts lined with epithelial cells surrounded by fibromuscular stroma [13, 14]. Homeostasis of normal prostate tissue is maintained by the crosstalk between epithelial cells and the surrounding stromal components [15, 16]. The glandular prostatic epithelium is a well-organized tissue composed of acini and ducts constituted by three types of cells, luminal, basal and neuroendrocrine cells (Figure 1). Luminal cells are columnar epithelial cells specialized in the production of prostatic secretions, including prostate specific antigen (PSA), and responsible for the main prostate function [17]. Basal cells adhere to the basement membrane and have the ability to produce several components essential in the maintenance of cell-growth [18, 19]. Neuroendocrine cells comprise less than 1% of the prostatic epithelium and express chromogranin A, synaptophysin, enolase 2, and CD56, which promote the growth of prostate [20]. Interactions between the epithelium and basement membrane are fundamental to maintain epithelial cell polarity involving apical and basal surfaces, which represent the well-differentiated cell state [13]. The non-epithelial tissue of the prostate, referred to as stroma, is composed essentially, by fibroblasts, smooth muscle cells and extracellular matrix (ECM) proteins (Figure 1) [15]. The ECM forms a dynamic and structured mixture of collagens, proteoglycans, thrombospondin, and hyaluronic acid, that respond to tissue injuries and allow its regeneration [16].

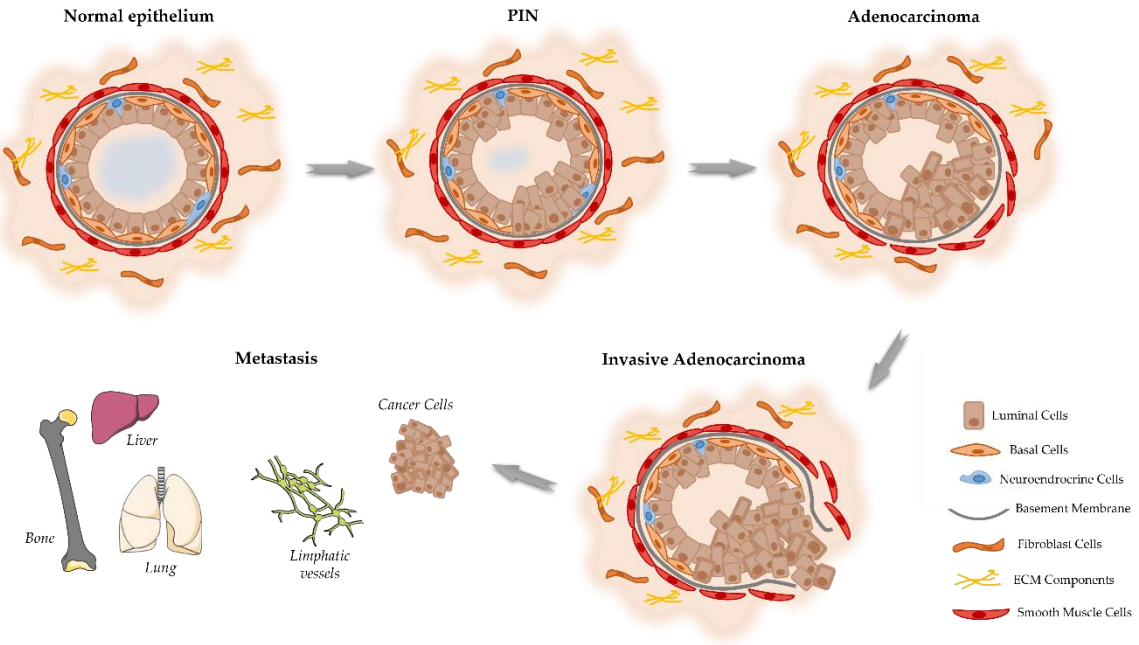

Figure 1. Schematic representation of the proposed model of the cellular events associated with the development and progression of PCa. Prostate epithelium is composed by the luminal cells responsible for the production of prostatic secretions, basal cells that are on the base of epithelium in contact with the basement membrane. Located among the epithelial cells also exist neuroendocrine cells that are involved in the regulation of secretory activity and prostate cell growth. Prostate epithelial cells maintain contact with the stroma, including smooth muscle cells, fibroblast cells and components of the extracellular matrix (ECM). Damage in the prostate normal epithelium induces the development of pre-neoplastic lesions called prostatic intraepithelial neoplasia (PIN). This stage progresses to localized prostate adenocarcinoma where the basal cell layer is lost, which then becomes invasive adenocarcinoma when the basement membrane is degraded, and neoplastic cells can invade to lymphatic system and other organs including liver, lungs and bones.

Considering the onset of PCa, there is a good agreement that this cancer develops from prostate epithelial cells [14]. However, conflicting evidence exists regarding if the oncogenic transformation in PCa arises from basal [19, 21] or luminal epithelial cells [22, 23]. In addition, it also has been hypothesized that PCa arising from luminal cells are more aggressive than those arising from basal cells [21]. The prostatic epithelium can be damaged and driven the carcinogenesis of prostate due to several factors, such as, inflammation, infections, genetic/epigenetic changes, persistent activation by androgens, exposure to carcinogens and/or genetic factors [14, 24]. The first identifiable histologic alteration in prostate malignant transformation is so-called prostatic intraepithelial neoplasia (PIN) (Figure 1) [25]. PIN lesions can be divided into two grades, low-grade PIN (LGPIN) and high-grade PIN (HGPIN), being that HGPIN lesions are considered the most likely precursors of PCa [26, 27], but they do not appear to raise serum PSA concentration [28]. Characteristically, HGPIN lesion contain basal cell layer around their periphery, although it is thin and often discontinuous. This is an important diagnostic feature because preservation of the basal cell layer can help to differentiate PIN from prostatic adenocarcinoma in which the basal cells are absent [24, 29].

Prostatic adenocarcinoma mostly arises in the peripheral zone of the prostate and initially is represented as a small foci of intraductal dysplasia, that with time differentiates and progresses into an invasive adenocarcinoma (Figure 1) [30]. The tumor foci lead to a disruption of prostate tissue and a decrease on glandular activity and prostatic fluid production [31]. Histologically, PCa is characterized by the destruction of the basal cell layer, derangement of the basement membrane, decreased epithelial cell polarity, and lack of

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connection of the glandular acini formed by the prostate epithelial cells [32]. As the tumor progresses, neoplastic cells increase the production of proteolytic enzymes, which cause degradation of the basement membrane, allowing the spread to adjacent tissues and the development of a metastatic disease [33]. Firstly, to lymph nodes and then to distant organs, including the bones, liver, and lungs, with bone as the most common site of metastasis [34]. In fact, in the context of epithelial neoplasia, the prostate stroma induces alterations in the tumor microenvironment, it is the so-called the reactive stroma. This phenotypic histological change leads to a loss of well-differentiated smooth muscle cells, increase of fibroblast population, and increase of secretion and deposition of ECM components, such as matrix metalloproteinase (MMP). All these changes can lead to epithelial cell depolarization and formation of conduits favoring neoplastic cell migration [16, 35]. All these histological changes cause a thousand-fold increased release of PSA from prostate neoplastic cells into the blood [32].

Androgens play a central role in the control of normal prostate as well as PCa cell growth and proliferation [14]. Androgens are the primary regulators of the proliferation/apoptosis ratio, stimulating proliferation and inhibiting apoptosis of prostate cells, and, thus, inducing the development of PCa [14, 36]. The major circulating androgen, testosterone, can be converted into DHT by the activity of 5α-reductase enzyme. Both testosterone and DHT exert their actions through binding to the AR. PCa growth and disease progression is initially dependent on AR activation. The main mechanism of action leads to the nuclear translocation of the ligand-receptor complex and subsequent binding to the androgen response elements (AREs), which initiates the transcription of genes that regulate cellular differentiation, proliferation and apoptosis (Figure 2) [27, 36, 37].

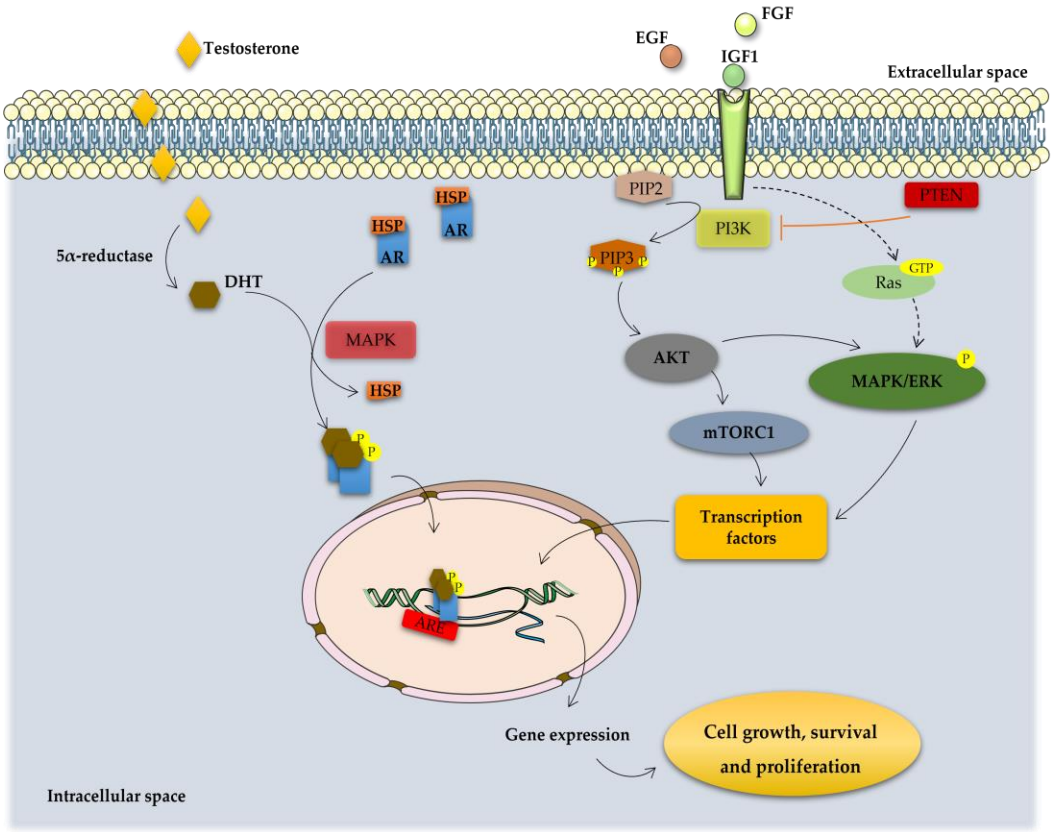

Figure 2. Overview of the molecular pathways associated with the development of CRPC. In the cytoplasm, activity of AR is regulated by ligand-binding and heat shock proteins (HSP). Testosterone is transported into the cytoplasm of androgen-receptive cells and is converted to 5α-dihydrotestosterone (DHT) by the enzyme 5α-reductase. DHT binding leads to dissociation of AR from

HSP and its phosphorylation by the mitogen-activated protein kinase (MAPK), which is followed by receptor dimerization and translocation into the nucleus where it binds to the androgen response elements (AREs) in the DNA activating transcription of genes essential for cell growth, survival and proliferation. On the other hand, PCa cell fate is controlled by receptor tyrosine kinases (RTK) activated by several growth factors, such as insulin-like growth factor (IGF1), fibroblast growth factor (FGF) and epidermal growth factor (EGF). RTK activation leads to the stimulation of phosphatidylinositol 3-kinase (PI3K) that phosphorylates phosphatidylinositol 4,5-bisphosphonate (PIP2) into phosphatidylinositol 3–5-triphosphate (PIP3). This process is inhibited by the tumor suppressor phosphatase and tensin homolog (PTEN). PIP3 activates, which subsequently removes the inhibition on the mTOR/Raptor complex (also known as mTORC1), thus leading to mTORC1 activation. mTORC1 is pivotal in the translation of proteins for protein synthesis and activation of transcription factors that translocate to the nucleus inducing the expression of pro-proliferation and anti-apoptotic genes. Other intracellular pathways also converge on the mTORC1 complex is constituted by the Ras-dependent pathway. Activated Ras (a small GTPase) phosphorylates and activates the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) cascade, regulating the activity of several transcription factors that are important for the cell cycle and proliferation. The activation of these signaling pathways inhibits apoptosis and induce the proliferation, invasion, and migration of PCa cells, being also implicated in tumor metastization.

In primary PCa, the action of AR keeps the same role as in normal prostate, for example, synthesis of PSA and modulating lipid metabolism [22]. However, it also triggers other events that promote epithelial cell growth, as the induction of the type II transmembrane serine protease (TMPRSS2):ETS fusion [26, 38]. The TMPRSS2 is an androgen-regulated gene overexpressed in PCa, which encodes a protein belonging to the serine protease family that functions in prostate carcinogenesis and relies on gene fusion with ETS transcription factors, such as ETS related gene (ERG) and ETV1. The TMPRSS2:ETS fusion is considered the most common chromosomal rearrangement in PCa and drives the overexpression of ETS oncogenes, previously identified as the most expressed proto-oncogenes present on malignant epithelial prostate cells [38–40]. ARs also have two active functional domains (AFs) that initiate transcription when activated. AF-1 is present in the NTD and its activation is androgen-independent. AF-2 is located in the LBD and is ligand-dependent [41]. AF-1 may enable cross-coupling between androgenic and growth factor signaling pathways [36, 42]. Therefore, these AFs are deemed clinically important as they could provide the key to understand the development of castration-resistant PCa (CRPC). At early stages of disease, PCa growth is androgen-dependent, the so-called androgen-sensitive PCa. However, with the continuous tumor development, PCa cells became androgen-insensitive, and the disease progresses to the so-called CRPC [36].

Patients that acquire resistance to the use of androgen-deprivation therapy (ADT) inevitably develop CRPC, a more lethal form of PCa. The role of AR in PCa progression and development of CRPC has been attributed to several factors, such as AR gene amplification, activating mutations and aberrant expression of co-activators [37, 43, 44]. These alterations lead to an increased AR expression, activation of AR by non-androgenic ligands, broadened ligand specificity and sensitivity and increased AR transactivation, which ultimately contribute to tumor cell growth in low androgen environment [36, 44, 45]. AR mutations in primary PCa are rare, but these mutations are prevalent in about 50% of CRPC [46, 47]. These mutations lead to alterations that improve the functional activity of the receptor, such as increased AR sensitivity to low levels of ligand, non-androgen ligand binding, ligand-independent activation as well as AR-independent pathways [41, 46, 47]. Furthermore, recent data indicate that an increased expression of constitutively active AR splice variants follows castration and are associated with poor prognostic and a rapid recurrence of PCa [48, 49]. The reduction in AR activation by endogenous androgen ligands leads to hypersensitization of other pathways of AR activation through ligand-independent mechanisms [44, 50].

Various growth factors, cytokines, kinases and other proteins have been shown to interact with and activate AR in a ligand-independent manner, including insulin-like growth factor (IGF1), fibroblast growth factor (FGF) and epidermal growth factor (EGF) [51, 52]. These growth factors activate tyrosine receptor kinases, which results in the activation of phosphatidylinositol 3-kinase (PI3K) and subsequently the PI3K/AKT pathway (Figure 2) [53]. The serine/threonine protein kinase (AKT), also known as protein kinase B (PKB), is one of the major downstream effectors of PI3K. Binding of ligands to the membrane growth factor receptors initiates a cascade of events that activate PI3K, which converts phosphatidylinositol 4,5-bisphosphonate (PIP2) to phosphatidylinositol 3–5-triphosphate (PIP3). PI3K activation stimulates AKT, which recruits proteins to the luminal cell cytoplasm [53, 54]. Downstream targets of AKT, namely, the mammalian target of rapamycin complex 1 (mTORC1), forkhead box protein O1 and the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) cascade, activate several transcription factors, such as c-myc, which induces the expression of proteins associated with cell survival and proliferation, cell cycle progression, migration and angiogenesis, and, thus, contributing to the progression of PCa [44, 53, 55].

3. Current use of chemotherapy in PCa

Treatment approaches for PCa differ depending on the stage of the disease. Several types of therapeutic options are available such as surgery, cryosurgery, radiation therapy, hormone therapy, chemotherapy, vaccine treatment, immunotherapy and bone-directed treatment [56]. Active surveillance is the recommended treatment option for low-risk PCa, monitoring its progression while not undergoing definitive therapy [57]. Therapeutic approaches based on surgery often are used in combination with therapeutic approaches based on drugs, namely hormone therapy and chemotherapy. Similarly to the non-neoplastic prostate cells, PCa cells need androgens to growth and survive, making the ADT an effective first-line therapy. This therapy can involve two approaches: surgical castration (i.e., orchiectomy) or, more commonly, chemical castration with drugs targeting AR signaling regulated by the hypothalamic pituitary gonadal axis (e.g., GnRH agonists, AR antagonists, and CYP17A1 inhibitors). This castration reduces tissue androgens levels and also reduce the expression of several androgen-regulated genes [34]. However, several adverse effects of ADT are known, such as decreased bone mineral density, metabolic changes, hot flashes, and sexual dysfunction [58]. Although most men show positive outcomes for 1 to 2 years with ADT, clinical progression occurs with the disease entering the stage of CRPC [36]. When PCa is considered castrate resistant different treatments options are needed, which includes chemotherapy [57]. This aggressive and lethal form of PCa progresses and metastasizes, not existing currently an effective therapy, being done only palliative care [59].

As the disease progresses to CRPC stage, treatment involves the use of chemotherapeutic drugs. Mitoxantrone was the first cytotoxic chemotherapy approved by FDA for metastatic PCa [60]. Next, other therapeutic agents for the treatment of CRPC were included, such as, the chemotherapeutic taxanes paclitaxel and docetaxel. After the discovery of the mechanism of action of paclitaxel, which is tubulin binding and enhanced microtubule polymerization resulting in mitotic arrest [61], other taxanes were explored and their synthetic and semisynthetic analogues with best properties and improved water solubility were produced [62]. The most successful semisynthetic analogue of paclitaxel is docetaxel, which is a taxane derivative that induces microtubules stabilization, arresting cells in the G2/M phase of the cell cycle, and it induces bcl-2 phosphorylation promoting a cascade of events that leads to apoptotic cell death (Figure 3) [63].

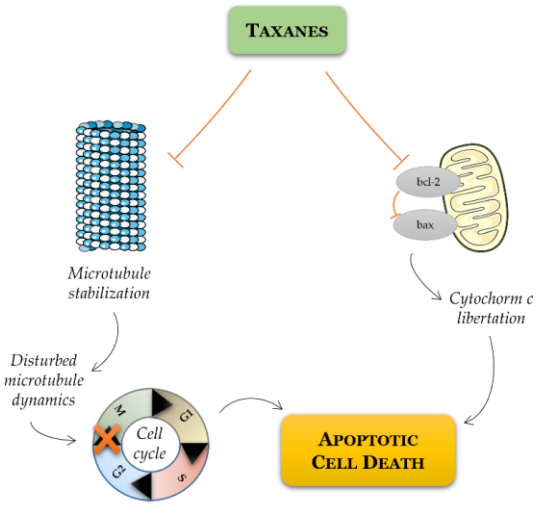

Figure 3. Schematic representation of mode of action of taxanes on cancer cell. Taxanes have been described to exert their antitumor efficacy via distinct modes of action: mitotic and apoptotic action. Taxanes bind to microtubules and thereby prevent their disassembly, resulting in G2/M cell cycle arrest and apoptosis. Alternatively, taxanes may inhibit the expression of antiapoptotic Bcl-2, favoring apoptotic cell death through the relief of BAX-mediated cytochrome c release.

Some studies using docetaxel as a single agent or in combination with other drugs showed objective response rates in up to 38% of patients, PSA declines in more than 50% of patients with hormone refractory PCa, and increased overall survival in metastatic PCa patients in approximately 24 months [60, 64, 65]. However, both paclitaxel and docetaxel drugs have a high affinity for multidrug resistance proteins [66]. Cabazitaxel is a novel third-generation semisynthetic analogue of docetaxel, and it is a promising treatment for docetaxel-resistant CRPC [67]. Like paclitaxel and docetaxel, cabazitaxel binds to tubulin and promotes its assembly into microtubules, while simultaneously inhibiting disassembly. This leads to the stabilization of microtubules, which results in the interference of mitotic and interphase cellular functions. The cell is then unable to progress further into the cell cycle, being stalled at metaphase, thus triggering apoptosis of the cancer cell [62]. In the last years, several studies have shown cabazitaxel as more effective in improving the life-quality of metastatic CRPC patients. Cabazitaxel induced molecular changes in favor of killing PCa cells when compared with other taxanes [68], showing a reduction of 30% of PSA levels in PCa patients [69], and cabazitaxel markedly improved the prognostic outcomes of metastatic CRPC patients [69, 70].

Multiple prospective randomized clinical trials have been designed to evaluate the efficacy and toxicity of therapies and diverse combinations have been attempted [71–73]. The CHAARTED (Chemohormonal Therapy versus Androgen Ablation Randomized Trial for Extensive Disease in PCa) and STAMPEDE (Systemic Therapy in Advancing or Metastatic PCa: Evaluation of Drug Efficacy) trials showed a remarkable overall survival benefit when combining ADT with docetaxel, as well as increased time to progression to castration resistant status [74, 75]. In the FIRSTANA (Cabazitaxel Versus Docetaxel Both With Prednisone in Patients With Metastatic CRPC) trial, cabazitaxel showed no superiority versus docetaxel for overall survival of PCa patients as first-line treatment [76]. Although the docetaxel and cabazitaxel have similar efficacy, they have different safety profiles, favoring the lower dose tested of cabazitaxel [77]. However, the CARD trial showed that high dose of cabazitaxel significantly improved a number of clinical outcomes, comparatively with the androgen-signaling-targeted inhibitor (abiraterone or enzalutamide), in patients with metastatic CRPC who had been previously treated with docetaxel and the alternative androgen-signaling-targeted agent (abiraterone or enzalutamide) [78]. These

results provide the evidence of a survival benefit with taxanes treatment in CRPC patients. Furthermore, patient preference studies have increased in significance in recent years for evidence-based medicine [79]. Therefore, the most recent clinical trial aimed to evaluate patient preference between docetaxel and cabazitaxel, the CABADOC trial [80]. This study showed a significantly higher proportion of chemotherapy-naïve men with metastatic CRPC who received both taxanes preferred cabazitaxel over docetaxel. Less fatigue and better quality of life were the two main reasons driving patient choice [80].

It is evident that the taxanes are constantly in upgrade both in terms of mechanistic and clinical aspects, and their success in treatment of PCa (castrate-sensitive and castrate-resistant settings) continued development of rational combination therapy strategies with the explicit goal to improve overall survival [73]. However, a persisting obstacle in taxanes administration is the ability of tumors to acquire resistance. This further opens the way for the exploration of new combinations to improve the efficacy and anticancer activity.

4. Transmembrane proteins as a potential therapeutic target in combination with taxanes

A transmembrane protein is a type of protein located either in the lipid bilayer of the plasma membrane or in the membrane of organelles [81]. Different from monotopic proteins, transmembrane proteins structure completely crosses the membrane [82]. Representing approximately 30% of the genome, transmembrane proteins are essential for many cellular processes [83]. These proteins are responsible for cell-cell and cell-environment communication, through signal transduction, the binding of receptors to hormones and neurotransmitters, and the transport of substances across the membrane [82, 83]. There are two types of transmembrane proteins regarding their structure, they are either alpha-helical proteins or beta-barrel proteins. They can also be categorized according to the protein topology, referring to the position of the N- and C-terminal domains [81, 82].

Several studies have shown a link between different transmembrane proteins and cancer, due their function related in cancer progression, metastasis, patient survival, and additionally, can also be used as therapeutic targets and/or biomarkers [81, 82]. Studies supporting the potential for targeting transmembrane proteins in taxane drug resistance in PCa are summarized in table 1.

Table 1. Identification of transmembrane proteins with combined effect of taxanes in PCa.

Protein	Function	Effect of knockdown alone	Effect of knockdown + taxane treatment
MDR1	Efflux pump	-	Improvement in docetaxel sensitivity
MRP4	Efflux pump	-	Resensitization to docetaxel treatment
CD44	Hyaluronate receptor	Reduced cell migration	Decrease viability of PC3 cells
CD133	Membrane organization	No alteration in cell proliferation and viability	Decrease in survival rate of cell, reduced metastatic potential, sensibilization to paclitaxel
SLCO1B3	Sodium-independent transporter	Reduction in cellular uptake of docetaxel	-
EGFR	Membrane receptor	Reduce cell proliferation	Tumor regression
STEAP1	Metalloreductase	Reduce cell viability and proliferation	Increase cell viability

4.1. MDR1

The efflux pump MDR1 (Multidrug Resistance Protein 1), also called p-glycoprotein, is a protein composed of 12 transmembrane domains and a single monomer of 170 kDa.

This protein is part of the ABC transporter family and is encoded by the *ABCB1* gene, located in the region 7q21 [84]. The overexpression of MDR1 is pointed out as partially responsible for drug resistance in PCa, due to higher drug efflux [85]. Regarding p-glycoprotein expression, Kawai *et al.* reported that both PCa and normal prostate epithelial cells are positive for the expression of the MDR1 gene. Using monoclonal antibodies to detect the presence of p-glycoprotein, the same study confirmed that this protein is asymmetrically expressed in the inner and outer zone of nonmalignant prostate glands. Moreover, the inner zone showed a higher level of protein expression [86].

To investigate whether the presence of p-glycoprotein in blood exosomes could be a marker to diagnose docetaxel resistance in PCa, Kato *et al.* tested the susceptibility to docetaxel and cabazitaxel drugs in parental and docetaxel-resistant PC3 cell lines considering p-glycoprotein expression. It was demonstrated that docetaxel-sensitive PC3 cells showed little or no expression of this protein, while docetaxel-resistant PC3 cells showed high expression of p-glycoprotein [87]. The knockdown of the *ABCB1* gene was also performed in docetaxel-resistant PC3 cells. The results indicated an improvement in docetaxel sensitivity when compared with the negative control. These findings confirm the relationship between p-glycoprotein expression and docetaxel resistance [87]. Additionally, another study on PC3, after demonstrating that the ETS1 transcription factor had a role in regulating the expression of the MDR1 gene, it was assessed how the downregulation of ETS1 could impact cell sensitivity to paclitaxel. Results showed that the combined treatment of paclitaxel exposure and knockdown of ETS1 induce a decrease in cell viability in paclitaxel-resistant PC3 cell line, improving the resistance to paclitaxel [88]. A further study using the C4-2B cell line, it was tested the association between the MDR1 protein and the retinoic acid receptor-related orphan receptor g (RORg) [89]. First, they established that MDR1 is regulated upstream by RORg, since the knockdown of the retinoic receptor decreased the expression of MDR1, while ectopic RORg increased it. Also, both RORg antagonists, SR2211 and GSK805, have led to the inhibition of MDR1 expression in taxane-resistant C4-2B cells [89]. Next, this study demonstrated that the knockdown alone and the use alone of RORg antagonist have led to a significant decrease in cell viability and growth in both taxane-resistant and not resistant C4-2B cell lines [89]. Furthermore, the combination between a partial RORg and a low concentration of docetaxel (20 nmol/L) led to a reduction of cell growth from 96,1% in control to 72,2% in treated cells. Likewise, the use of 1.25 mmol/L SR2211 combined with 12.5 nmol/L docetaxel reduces the viability of taxane-resistant C4-2B to 33,2%, suggesting that the downregulation of RORg can sensitize taxane-resistant CRPC cells to taxane treatment [89].

4.2. MRP4

Similarly to the MDR1 protein, the MRP4 protein, also known as multidrug resistance protein 4, is part of the ATP-binding cassette (ABC) transporters family [90]. This transmembrane protein is present in almost all tissues in the body, such as brain, kidney, liver, erythrocytes, platelets, adrenal gland, and pancreas [91]. MRP4 is responsible for the transportation of prostaglandins E1 and E2 (PGE1 and PGE2) as well as cAMP and cGMP [92]. The MRP4 protein was reported as being highly overexpressed in docetaxel-resistant C4-2B cells, while no expression of MRP4 was detected in docetaxel-sensitive C4-2B cells [93]. To assess if the overexpression of MRP4 leads to docetaxel resistance, combined treatment of MRP4 knockdown plus docetaxel exposure were given to docetaxel-resistant C4-2B cell line. The results showed a diminished cell viability, indicating a resensitization to docetaxel treatment [93]. Furthermore, researchers assessed the hypothesis that androgens are responsible for MRP4 overexpression in docetaxel-resistant cells [93]. For this, C4-2B cells were exposed to DHT or bicalutamide followed by the quantification of MRP4 mRNA and protein levels [93]. After treatment with DHT, both mRNA and protein levels were

increased, displaying a dose-dependent manner [93]. However, the exposure to bicalutamide prevented the upregulation of MRP4 [93]. This data shows that MRP4 can be upregulated by androgen and downregulated by anti-androgen treatment [93].

4.3. CD44

CD44 is a non-kinase cell surface transmembrane glycoprotein. This important hyaluronate receptor is overexpressed in cancer stem cells and is involved in cellular adhesion and communication, lymphopoiesis, myelopoiesis, and angiogenesis. In regard to cancer, CD44 is implicated in metastasis, cellular growth, proliferation, migration, and invasion [94]. There are several isoforms for the CD44 protein and some of them have been associated with PCa, namely the CD44s, CD44v6, and CD44v7-10 isoforms [94]. Furthermore, CD44 is also overexpressed in this type of cancer and is associated with aggressive biological behavior and a poor prognosis [94]. CD44 expression is upregulated by transforming growth factor-beta 1 (TGF-β1) in PCa cells [94]. CD44 is expressed in PC3 cells and was demonstrated that this receptor regulates glucose metabolism, intracellular reactive oxygen species (ROS), and cell proliferation in those cells; however, CD44 is not expressed in LNCaP cells [90]. Collected data also points to the regulation of proliferation, invasion, and migration via PDK1 and PFKFB4, which are enzymes that regulated glucose metabolism and are modulated by CD44 [95]. Li *et al.* reported that the use of docetaxel treatment combined with SB-3CT, a possible inhibitor of CD44 cleavage, decrease the viability of PC3 cells in comparison with the docetaxel-only treatment [95]. Researchers also assessed the combination index using CompuSyn software, showing that mild to moderate synergistic effects were observed for an SB-3CT concentration of 20μmol/L in combination with docetaxel [95]. Lai *et al.* also reported that docetaxel-resistant PC3 and DU145 cells have a higher migration and invasion rate than the parental cells [96]. In addition, when analyzed for the $CD44^+$ population, both docetaxel-resistant cell lines showed higher numbers than the parental cells [96]. The knockdown of CD44 reduced cell migration in both docetaxel-resistant cell lines, while invasion has been suppressed only in docetaxel-resistant PC3 cells [96].

4.4. CD133

The pentaspan transmembrane glycoprotein CD133, also known as prominin-1, is a protein mostly found in the microvilli of different epithelial cells but is also expressed in numerous types of cancer such as breast, ovarian, and PCa and other non-epithelial cell types [97, 98]. CD133 is frequently used as a biomarker for the detection of cancer stem cells [98]. The molecular function of this glycoprotein has not been yet fully clarified but there is strong evidence pointing towards a role in membrane organization, due to its preferred location on the microvilli, and a role in spermatozoa biogenesis and photoreceptor disc formation [97]. Regarding the photoreceptor disc formation, it is known that a mutation on the *CD133* gene is the cause of a type of macular degeneration called Stargard disease [97]. CD133 is also important in angiogenesis through the regulation of expression of vascular endothelial growth factor (VEGF) [97]. Concerning the expression of CD133 in PCa cell lines, flow cytometric analysis performed by Wang *et al.*, found that $CD133^+$ cells were only present in the DU145 cell line, and indetectable in PC3 and LNCaP cell lines, when cultured in normal conditions [99]. However, when cultured in a serum-free medium, the PC3 cell line was able to present an increased proportion of $CD133^+$ cells [99]. In LNCaP cells, the presence of $CD133^+$ remained not observable. Nonetheless, Aghajani *et al.* evaluated the CD133 mRNA expression levels in the same PCa cell lines and discovered that CD133 is low expressed in all three cell lines, although with higher expression levels in the LNCaP cell line [100]. Additionally, Wang *et al.* assessed the possibility of enriching the proportion of $CD133^+$ cells via chemotherapy, for which a docetaxel-containing medium was used in DU145 cell culture [99]. An increase of 9.8% in the proportion of $CD133^+$ cells was observed after treatment, corroborating that those cells are chemo-

resistant [99]. Through studying the knockdown alone of CD133 and in combination with paclitaxel, Aghajani *et al.* reported that, in LNCaP cells, the downregulation alone did not alter cell proliferation and viability when compared to the control group [100]. However, the combination with the paclitaxel treatment led to a decrease in survival rate compared to the LNCaP cells that were uniquely treated with paclitaxel. Regarding the migration and invasiveness, both knockdown of CD133 or paclitaxel alone treatment was able to reduce it, while the combination of treatments led to a synergistic decrease [100]. Also, the combination CD133-siRNA/paclitaxel significantly reduced the metastatic potential due to a lower expression of vimentin and MMP9 [100]. Finally, an apoptosis study using the LNCaP cells showed that the knockdown of CD133 may increase the sensitivity to paclitaxel [100].

4.5. SLCO1B3

Belonging to the Solute Carriers superfamily, SLCO1B3, also called organic anion transporting polypeptide (OATP) [101] is a sodium-independent transporter of both endogenous substrates such as bilirubin, bile salts, steroid conjugates, bromosulfophthalein (BSP), Taurocholate (TCA) [101, 102] as well as exogenous substrates as antihistamines, blood-glucose-lowering drugs, statins, heart medications, and also docetaxel and paclitaxel [101, 103].

Konig *et al.* confirmed that, under normal conditions, SLCO1B3 is exclusively expressed on hepatocytes, with its subcellular location on the basolateral plasma membrane of those cells [104]. Additionally, a preferred lobular zonation was also observed, where the hepatocytes near the central vein had a higher expression of this protein when compared to other locations within the liver [104]. Meanwhile, several studies have confirmed the abnormal expression of SLCO1B3 in tumorous tissue, including PCa [105]. Wright *et al.* demonstrated a significantly higher expression of the gene SLCO1B3 in CRPC metastases in comparison to untreated primary PCa [101]. In addition, a higher risk for PCa-specific mortality was connected to the SNP (Single nucleotide polymorphism) SLCO1B3 rs4149117 [106]. Moreover, SLCO1B3 mRNA levels was found in 62% of the prostate tumor samples, but no expression was detected in normal prostate [103]. The same study also indicated a clear positive association between the Gleason score and SLCO1B3 expression [103].

Regarding the effects of taxanes, a study evaluated patient-derived xenografts (PDXs) of PCa and discovered that docetaxel-resistant PDX tumors presented a significant downregulation of SLCO1B3. Along with this result, the PDXs presented reduced intratumorally docetaxel concentrations. To assess if the downregulation of SLCO1B3 was responsible for the low concentration of docetaxel, the silencing of SLCO1B3, as well as other docetaxel transporters, was performed. Only cells that were transfected with the SLCO1B3 siRNA presented a significant reduction in docetaxel uptake. To further investigate the role of SLCO1B3, SLCO1B3-negative PDXs were transfected with SLCO1B3 and later exposed to docetaxel and cabazitaxel. The outcome pointed toward a higher sensitivity to both taxanes drugs treatments among SLCO1B3-overexpressing cells [107].

4.6. EGFR

The transmembrane glycoproteins epidermal growth factor receptor (EGFR) together with HER-2/neu (erbB-2), HER-3 (erbB-3) and HER-4 (erbB-4), belongs to the HER (erbB) family of membrane receptors [108]. All these receptors are expressed in both normal and malignant cells, playing important roles in cell proliferation and differentiation [109]. All four family members have a very alike structure, consisting of three regions: an extracellular ligand-binding region, which, in the case of EGFR, is the binding region for the epidermal growth factor (EGF), transforming growth factor-a (TGF-a), amphiregulin (AR), Heparin-binding EGF-like growth factor (HB-EGF), and betacellulin (BTC) [108,

109]. HER2 dimerizes with EGFR [110] and has no exclusive natural ligand [108]. The second region, a transmembrane domain, consisting of a single hydrophobic anchor sequence that crosses the cell membrane only once [109]. Lastly, acting as a binding site for intracellular substrates, and therefore, activating signaling pathways. The intracellular domain has tyrosine kinase activity [108]. Rossini *et al.* confirmed that DU145 and PC3 cell lines express the activated form of the EGFR and HER-2 receptors [111]. LNCaP and C4-2B cell lines also express EGFR, being higher in C4-2B cells [112]. In *in vivo* studies, EGFR was confirmed as overexpressed in both metastatic and CRCP, as well as moderately expressed in localized primary PCa. Furthermore, the assessment of EGFR expression on circulating tumor cells from the blood of patients with metastatic disease demonstrated that 90% of patients presented circulating tumor cells positive for EGFR [112]. Vicentini *et al.* studied the use of ZD1839, a selective EGFR tyrosine kinase inhibitor, in both androgen-sensitive cell lines (ND1, LNCaP and ALVA-31) as well as androgen-independent cell lines (PC3, DU145 and TSU-Pr1) [113]. First, it was reported by the authors that higher levels of EGFR and its ligands were present in the androgen-receptor-negative cell lines. However, ZD1839 treatment resulted in reduced cell proliferation in all cell lines tested [113]. Furthermore, a *in vivo* study assessed the tumor mass response to the blockade of EGFR and HER2 [111]. In order to do that, subcutaneous DU145 or PC-3 tumors were established on male mice, and tumor volume was quantified before, during, and after treatments [111]. It was demonstrated that Cetuximab and Trastuzumab (blockers of EGFR and HER2, respectively) in combination with docetaxel treatment induced a significant tumor regression when compared to the respective control group [111]. Furthermore, 80% of mice that were given the triple combination end up tumor-free. However, even though docetaxel alone, cetuximab alone, and in combination with Trastuzumab showed significant tumor growth inhibition, tumor regrowth was observed [111]. In another study, Monteverde *et al.* used the tyrosine kinase inhibitor Vandetanib to target EGFR in sensitive and docetaxel-resistant PC3 cell lines [114]. The study showed that the docetaxel-resistant PC3 cells present 3 times the amount of EGFR mRNA and 12 times the amount of EGFR protein when compared to sensitive PC3 cells. Additionally, the treatment with docetaxel alone produced an increase in the pEGFR/EFGR ratio, while the combination with Vandetanib had the opposite effect [114]. In docetaxel-resistant PC3 cells, no treatment altered the pEGFR/EFGR ratio. Regarding the effect in cell proliferation, a maximum of 90% inhibition was observed in response to docetaxel treatment alone in both sensitive and resistant PC3 cells. To attain this inhibition, it was necessary a 2×10^{-9} M concentration of docetaxel for the sensitive cell line and a 0.9×10^{-7} M concentration for the resistant. Vandetanib alone also displayed inhibition effects but the strongest cytotoxic effect were observed when vandetanib was combined with low concentrations of docetaxel (0.061– 0.246 nM), for which the combination index value is 0.49–0,71. However, for resistant PC3 cells, there were different results regarding if the treatment was administrated in sequence (vandetanib followed by docetaxel) or together. In the first case, the combination index value of 0.55–0.90 indicated a synergetic effect, but for the treatment when given together a combination index of 1.22–1.73 was found, indicating a possible antagonism [114].

4.7. STEAP1

STEAP1, together with STEAP2-4, is part of the six-transmembrane epithelial antigen of prostate (STEAP) family of proteins [115]. The STEAP1 protein is overexpressed in several human cancers, including prostate, bladder, colon ovary, breast, and cervical cancer [116]. Although its function remains unclear, some studies have pointed out that STEAP1 is involved in metal reductase activity, and also in transport of ions such as Na^+, Ca^{2+}, and K^+ [117]. STEAP1 is highly expressed in LNCaP cells and also at significant levels in C4-2B cell line [118]. Regarding the effect of STEAP1 knockdown in LNCaP cells, reduced cell viability was observed in comparison to the control group. This result was supported by the cell proliferation index, showing a 0.3-fold decrease in LNCaP cells

knocked down for STEAP1. Besides the effect in inhibition of cell proliferation, the STEAP1 knockdown increased the number of apoptotic cells [119]. The same study also evaluated the behavior of LNCaP cells knocked down for STEAP1 in response to DHT, and the result was that the effect of *STEAP1* gene silencing was not reversed after exposure to DHT [119]. Recently, another study reported the effect of paclitaxel, docetaxel and cabazitaxel on STEAP1 expression in LNCaP and C4-2B cells [118]. It was observed that paclitaxel or cabazitaxel treatment increased the STEAP1 protein expression when compared with the control group, but no differences were observed in C4-2B cells [118]. Furthermore, it was reported that STEAP1 knockdown alone decreased the cell viability in both cell lines, as well as all taxane-based treatments when administered alone. However, the combination between STEAP1 knockdown and exposure to taxane-based therapy led to an increase in cell viability/proliferation and diminished levels of apoptosis [118]. Altough more studies are required, these data suggest that the combination of taxane-based drugs with STEAP1 knockdown may lead to PCa progression.

5. Conclusions

Taxanes based-chemotherapeutic drugs are currently the main approach when it comes to PCa treatment. Even though this type of therapy has good results in improving patient survival, the development of resistance to chemotherapeutic drugs remains a great obstacle. In this review, we have covered state-of-the-art on the use of taxane-based therapy combined with targeting different transmembrane oncoproteins in PCa. The knockdown of transmembrane oncoproteins can improve, in some cases, taxane sensitivity, and therefore, might be a mechanism to improve the efficacy of taxanes drugs. However, it should be taken into account that some combinations may even trigger harmful effects, such as the knockdown of STEAP1. Besides the proteins described in this article, there are much more transmembrane oncoproteins whose specific role in PCa and association with taxane resistance requires further elucidation.

Despite some studies have been shown a promising use of taxane treatment in combination with inhibitors of transmembrane oncoproteins, additional studies are still needed to support a translation for clinical practice. Most of the scientific studies are focused in cell lines, which present several limitations. Therefore, it is required to perform studies using animal models in order to find good combinations to evaluate in clinical trials.

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