Chapter 13
TCTP Has a Crucial Role in the Different Stages of Prostate Cancer Malignant Progression

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Abstract Prostate cancer (PC) is the second most common cause of cancer-related mortality in men in the western world after lung cancer. Many patients are not candidates for resection given the advanced stage of their cancer. The primary treatment for advanced PC is the castration therapy which supresses the production of androgens, hormone that promotes PC growth. Despite the efficiency of the castration therapy, most patients develop castration resistant disease which remains uncurable. Clearly, novel approaches are required to effectively treat castration resistant PC (CRPC). New strategies that identify the molecular mechanisms by which PC becomes resistant to conventional therapies may enable the identification of novel therapeutic targets that could improve clinical outcome. Recent studies have demonstrated the implication of TCTP's over-expression in PC and CRPC, and its role in resistance to treatment. TCTP's interaction with p53 and their negative feedback loop regulation have also been described to be causal for PC progression and invasion. A novel nanotherapy that inhibits TCTP has been developed as a new therapeutical strategy in CRPC. This chapter will highlight the role of TCTP as new therapeutic target in PC, in particular, therapy-resistant advanced PC and report the development of novel nanotherapy against TCTP that restore treatment-sensitivity in CRPC that deserve to be tested in clinical trial.

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13.1 TCTP Is Upregulated in Prostate Cancer

Although Prostate cancer (PC) is a deadly cancer with a rapidly increasing frequency in the western countries localized PC is usually treated with surgery and radiation (American cancer society: prostate cancer statistics http://www.cancer.org) (Jemal et al. 2010; Siegel et al. 2012). The importance of androgens for the initiation and progression of PC has been shown early in the twentieth century. Androgens bind to their specific receptors (AR) and are well known to supply the PC cell growth.

In 2004, a first study has shown that, while TCTP appears to be essential for prostate gland normal physiologic functions, its expression is increased in PC cells (Arcuri et al. 2004). The authors described TCTP as one of the main calcium binding protein in PC cells in which it regulates key processes like apoptosis and cellular differentiation. More recently, Gnanasekar et al. have examined if TCTP was a good potential new therapeutic target in androgen-dependent PC cells. Specifically, it has been demonstrated that silencing TCTP gene with a siRNA dramatically increases the androgen-sensitive PC cell death due to apoptosis (Gnanasekar et al. 2009). TCTP has further been identified as a novel androgen regulated gene whose expression is induced at both mRNA and protein level by androgens (Kaarbo et al. 2013).

13.2 TCTP: A Promising Target in Castration-Resistant Prostate Cancer

The first-line treatment for advanced or metastatic PC is the castration therapy consisting in androgen deprivation. Castration therapy cut off the supply of androgens that encourage PC growth (McLeod 2003; Theodore 2004). Despite the dramatic tumor regression that follows the castration therapy, the patients will ultimately become unresponsive and the prostate tumors will relapse within 1–3 years in a more aggressive castration-resistant mode (Fusi et al. 2004).

13.2.1 TCTP Mediates Heat Shock Protein 27 Cytoprotective Function in CRPC

In 2005, Rocchi et al. found that one of the most common genetic events in castration-resistant prostate cancer (CRPC) is the activation of heat shock protein 27 (Hsp27) expression (Rocchi et al. 2005). But the mechanism by which Hsp27 induces a multi-drugs resistance to CRPC tumors was unknown. Thus, in order to elucidate the pathways by which Hsp27 imposes its cytoprotective effect and find new therapeutic targets specific of CRPC tumors, Dr. Rocchi laboratory has

screened all Hsp27 interactors using a two-hybrid system (Katsogiannou et al. 2014) and identified TCTP as a new Hsp27 protein partner (Baylot et al. 2012). This work pinpoints for the first time TCTP as a potential therapeutic target in CRPC (Baylot et al. 2012; Acunzo et al. 2014).

This study has demonstrated that TCTP protein level correlates with PC cells aggressiveness. In castration-resistant (CR) prostate tumor cells, TCTP protein expression is strongly increased compared to its expression in castration naïve PC cells. Furthermore, a tissue microarray experiment performed on 211 clinical specimens showed that TCTP is highly uniformly overexpressed in 75% of the CRPC samples. These observations highlight its association with the aggressiveness of the human disease. Additionally, no or weak TCTP expression has been detected in normal or benign tissues, suggesting that targeting TCTP in human CRPC may cause only weak undesirable toxicity in normal tissues.

Further mechanistic investigations showed that in castration-sensitive cells, overexpressing Hsp27 is sufficient to increase TCTP protein level but not TCTP mRNA level. Additionally, it has been demonstrated that Hsp27 is a direct upstream regulator of TCTP and that this chaperone protects TCTP from its ubiquitination and proteasomal degradation.

In CRPC cells, TCTP inhibition leads to cell viability reduction, cell cycle arrest, and caspase-*3*-dependent apoptosis activation. Moreover, in castration naïve PC cells stably overexpressing Hsp27, TCTP downregulation increases apoptosis *via* caspase-*3* activation and enhances chemotherapy. These data show that TCTP silencing suppresses the chemo-resistance of CRPC cells due to high Hsp27 levels and suggest that TCTP is a mediator of Hsp27 cytoprotective function in CR prostate tumors. Furthermore, targeting TCTP in vivo with an antisense oligonucleotide, developed by Dr. Rocchi laboratory (Baylot et al. Patent PCT10306447.3 2010), suppresses the growth of PC cell xenografts and significantly enhances chemotherapy activity upon systemic delivery. These findings open up the possibility for using TCTP knockdown in combination with other established therapeutic approaches to increase treatment efficacy in CRPC.

13.2.2 TCTP and P53 in CRPC: "Neither Can Live While the Other Survives"

Very interestingly, Baylot et al. also found that CR progression correlates with the loss of the tumor suppressor P53. In a prior study, Amson et al. has shown that TCTP and P53 are involved in a reciprocal negative-feedback loop in breast cancer (Amson et al. 2012). But the role of P53 in the PC progression was still elusive. Baylot et al. has demonstrated for the first time a link between TCTP, P53, and the CR progression of PC (Fig. 13.1).

On one hand, after castration, the prostate tumors that have progressed from a castration-sensitive state to a CR state overexpress TCTP and loose P53 expression.

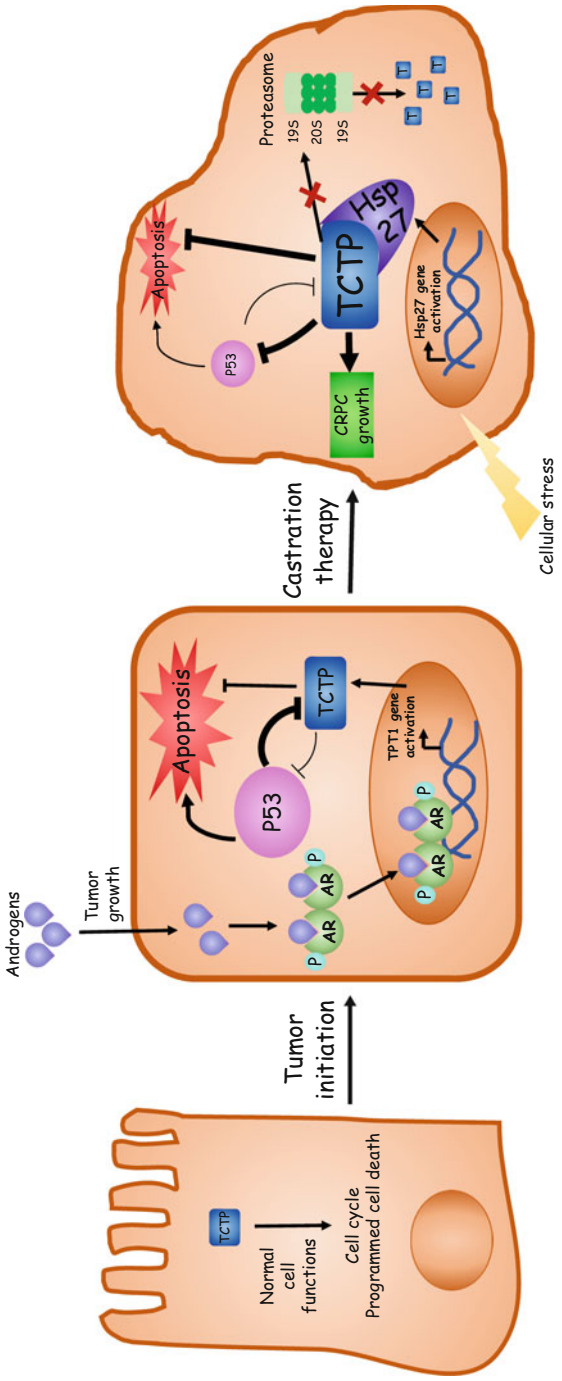

Fig. 13.1 The central role of TCTP in Prostate Cancer (PC) progression. Despite the essential role of TCTP in normal prostate epithelial cells (*first panel*), TCTP expression is highly increased in prostate tumor cells (*second and third panels*). In castration-sensitive PC (CSPC), the androgen receptor (AR) directly activates the transcription of TCTP by binding to the promoter of this gene (TPT-1). In early stages of PC, the tumor suppressor P53 is highly expressed and represses TCTP transcription. These opposite functions of the AR and P53 maintain a low level of TCTP in CSPC leading to a high sensitivity of the CS tumors to therapies-induced apoptosis (*second panel*). Following the castration therapy, the tumors will initially regress but within 1 to 3 years they will relapse in a castration-resistant mode. In castration-resistant PC (CRPC), heat shock protein 27 (Hsp27) is highly expressed and its cytoprotective function is crucial for CRPC growth, invasion, and chemo-resistance. By directly binding to TCTP protein in the cytoplasm of CRPC cells, Hsp27 protects TCTP from its degradation by the ubiquitin–proteasome pathway, leading to a massive increase of TCTP protein level in CRPC tumors. In late stages of PC, the high level of TCTP protein level promotes P53 degradation and therapies-induced apoptosis resistance (*third panel*)

TCTP silencing using TCTP antisense oligonucleotide is able to restore P53 expression and function in CRPC tumors, suggesting that castration sensitivity is directly linked to P53. On the other hand, P53 downregulation in castration-sensitive PC cells significantly inhibits chemotherapy-induced apoptosis compared to the control cells, suggesting an important link between P53 status and PC tumors chemotherapy resistance.

These data show that TCTP is upregulated in CRPC tumors leading to the loss of P53 expression and function together with castration- and chemo-therapies resistance. This work importantly highlights the crucial role of TCTP/P53 axis in CR progression of PC.

13.2.3 Development of a TCTP Antisense Oligonucleotide for Clinical Applications

As mentioned above, Dr. Rocchi laboratory has developed a TCTP inhibitor that can be used for human therapy and has screened by gene walk all antisense oligonucleotide (ASO) sequences targeting TCTP full-length mRNA (Karaki et al. 2017). Initially, 28 ASOs have been designed. Finally, three ASO lead sequences, that potently inhibited TCTP expression, have been furthered examined for their ability to affect CRPC cells and tumor growth. Thus, it has been reported that TCTP-ASOs enter to the cells *via* macropinocytosis, increased caspase-*3*-dependent apoptosis, blocked cell cycle, and enhanced chemotherapy in CRPC cells in vitro. And, consistent with these in vitro data, systemic administration of TCTP-ASOs in immunocompromised mice suppressed CRPC tumor growth and also significantly enhanced castration and chemotherapy activities in vivo. Additionally, TCTP-ASO treated mice showed a significant decrease of Ki-67 levels, a proliferation marker, compared to the control group. Moreover, possible toxic effects resulting from oligonucleotide administration have been checked. The animals did not show any change neither in their behavior, nor in their body weight during the experiment. Furthermore, treated mice did not show any signs for hepatic damage, since their aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were within the range of normal values, and they didn't show significant difference compared to the control group. Finally, no sign of renal damage was observed, since the creatinine levels were normal and the biochemical analyses of urine were normal for the tested parameters.

13.3 Discussion

Taken together, these studies demonstrate that TCTP has a crucial role in the different stages of PC malignant progression from the tumor initiation to the multi-drugs resistant stage of the disease.

Arcuri et al. were the first to investigate TCTP expression and function in human prostate normal and cancer tissues. The authors have notably found that TCTP is the most highly expressed calcium binding protein in the human prostate cancer cells. Subsequent studies from different laboratories confirmed that TCTP is upregulated in early stages of the disease, in which the prostate tumor growth is fueled by androgens (Gnanasekar et al. 2009; Kaarbo et al. 2013). TCTP has since been identified as an androgen-regulated gene (Kaarbo et al. 2013). Furthermore, TCTP has been reported to be causal for the resistance to androgen withdrawal and chemotherapy in PC (Baylot et al. 2012; Acunzo et al. 2014). This work has validated, using 211 clinical specimens, that TCTP is slightly over-expressed in the castration naïve specimens compared to normal specimen, confirming his implication in PC initiation, and that its expression is abolished upon castration therapy. Most importantly this study has shown for the first time, that TCTP is highly over-expressed in multi-drugs resistant prostate tumors and metastases, pinpointing TCTP as a key protein in the late stages of PC in which the tumors grow in a castration-resistant mode (CRPC). Currently, there is no effective therapy for patients with CRPC and existing novel therapies only have a modest impact on the overall survival of these patients (McKeage 2012; de Bono et al. 2010). Clearly, novel approaches were required to effectively treat CRPC, in particular new strategies that identify the molecular mechanisms by which CRPC becomes chemo-resistant, as well as the identification of novel therapeutic targets that could improve clinical outcome. Thus, identifying TCTP as a new therapeutic target for the treatment of CRPC represents a major advance in the field. Additionally, altogether these findings also strongly suggest that TCTP is highly prognostic in human PC.

A TCTP inhibitor has been developed by Dr. Rocchi laboratory (Baylot et al., Patent PCT10306447.3; 2010) for clinical applications. Recent results showed that the TCTP inhibitor can suppress CRPC tumor growth and enhance castration- and chemo-therapies in vitro and in vivo. The stability, biodisponibility, and delivery improvement of this TCTP inhibitor for human treatment is currently under investigation in Dr. Rocchi laboratory and represents today a great hope for the patients with CRPC.

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