

Review Article

Protein tyrosine kinase 6 signaling in prostate cancer

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Abstract: More than 25 years have passed since the discovery of protein tyrosine kinase 6 (PTK6), a non-receptor tyrosine kinase distantly related to SRC family kinases. Since then, a variety of data suggest that PTK6 promotes oncogenic signaling and tumorigenesis, generally dependent on its kinase activity. Increased PTK6 expression, activation at the plasma membrane and altered intracellular localization have been discovered in prostate cancers. While PTK6 is localized to nuclei of epithelial cells in normal prostate, it is relocalized and activated at the plasma membrane in prostate tumors. Active PTK6 interacts with and directly phosphorylates AKT, FAK and BCAR1 to promote oncogenic signaling. Furthermore, PTK6 can enhance the epithelial mesenchymal transition by inhibiting E-cadherin expression and inducing expression of the mesenchymal markers vimentin, SLUG and ZEB1. Several lines of evidence suggest that PTK6 plays a role in *Pten* null prostate tumors. PTEN targets activating phosphorylation of PTK6 and loss of PTEN subsequently leads to PTK6 activation. Different studies provide compelling evidence as to why PTK6 is a potential therapeutic target in prostate cancer. Here, we briefly review the advances and significance of PTK6 in prostate cancer.

Keywords: PTK6, BRK, PTEN, tyrosine kinase, prostate cancer

Introduction

Prostate cancer is the second leading cause of cancer related deaths among men in The United States, with an estimated 191,930 new cases and 33,330 related deaths in 2020 according to the American Cancer Society [1]. It is also estimated that by 2030, the burden of the disease will approach 1.7 million new cases and 499,000 deaths worldwide. While the mortality rate of prostate cancer is considered low, with nearly 100% 5-year survival rate in localized tumors, the risk of metastasis related deaths is high leading to a decrease in the 5-year survival of patients to 31% [1]. Molecular mechanisms underlying prostate cancer initiation and progression include NKX3.1 downregulation, MYC-upregulation, persistent androgen receptor activity, TMPRSS2-ERG translocation, *PTEN* deletion, activation of PI3K and MAPK signaling, upregulation of Ezh2, miRNAs, and oncogenic activation of both membrane and intracellular tyrosine kinases [2, 3]. Castration resistant prostate cancer (CRPC) which has developed resistance to anti-androgen therapies is a major cause of lethality.

Tyrosine kinases, including receptors and intracellular kinases, play major roles in prostate cancer signal transduction. They regulate cell differentiation, cell cycle progression, adhesion and metastasis. The protein tyrosine kinase 6 (PTK6) family of non-receptor tyrosine kinases, including PTK6, FRK and SRMS, is distantly related to SRC family of kinases. SRC family kinases contain four domains essential for their function, including the SRC homology (SH) 4 domain that is required for lipid modification and membrane localization, and the SH3 and SH2 domains that mediate protein-protein interactions and kinase autoregulation. PTK6 family kinases do not contain SH4 domains required for lipid modification and are not targeted to any specific cellular compartment (reviewed in [4]). Members of the PTK6 family share a distinct gene structure that is different from other intracellular tyrosine kinases [5, 6]. PTK6 (also known as BRK; Breast Tumor Kinase), represents the most extensively studied member of the PTK6 family.

It should be noted that expression levels of PTK6 do not necessarily correspond with

PTK6 in prostate cancer

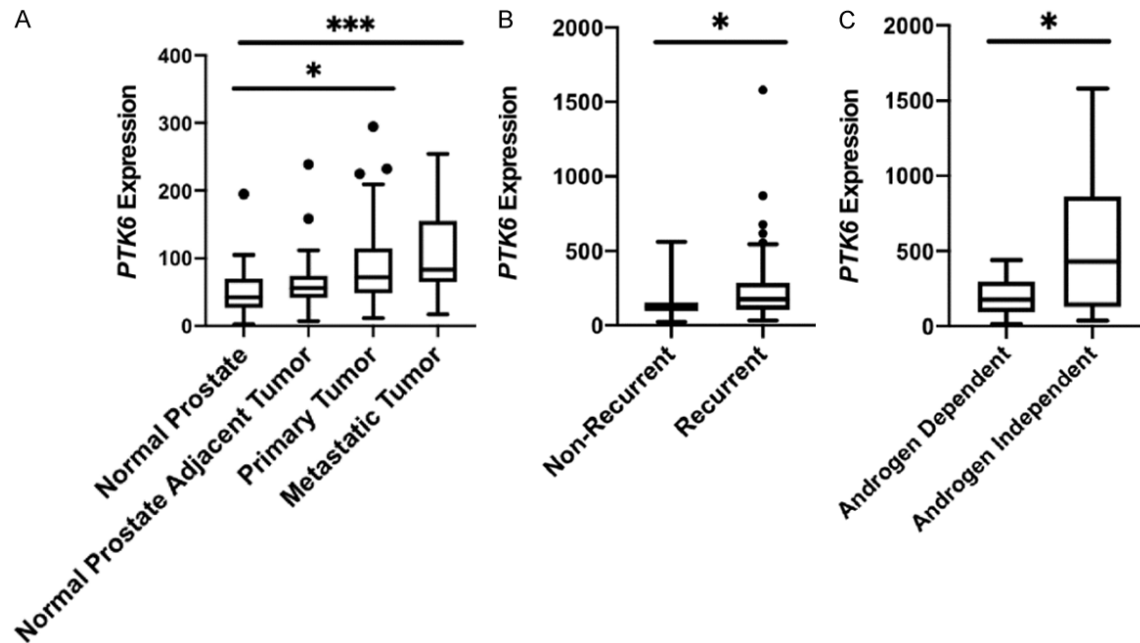


Figure 1. *PTK6* expression correlates with prostate cancer progression. Analysis of public datasets from NCBI human genome microarray (GEO) website reveals an increase in *PTK6* mRNA expression in multiple datasets. A. Primary (N=65) and metastatic prostate tumors (N=25) exhibit higher *PTK6* mRNA compared with normal (N=25) and normal adjacent tumor prostate (N=56) (dataset GDS2545 [57], also described in [11]). B. Analysis of dataset GDS4109 [58] indicates that Recurrent (N=40) tumors express higher *PTK6* mRNA than non-recurrent prostate tumors (N=39). C. By analyzing dataset GDS1390 [59], we found that patients with androgen independent tumors (N=10) have higher *PTK6* mRNA levels than patients with androgen dependent tumors (N=10), suggesting that *PTK6* mRNA levels positively correlates with androgen independence. (*) represents $P < 0.01$; (***) represents $P < 0.001$.

its kinase activity. PTK6 has both signaling and adaptor functions that are mediated through protein-protein interactions involving its SH3 and SH2 domains. Like SRC-family kinases, PTK6 family kinases are regulated by phosphorylation. PTK6 is activated when phosphorylated at tyrosine residue 342, while it is negatively regulated by phosphorylation at tyrosine residue 447 [7], which could be mediated by the PTK6 family member SRMS [8].

PTK6 expression and activation in prostate cancer

PTK6 is primarily expressed in epithelial linings, with highest levels in the intestine and skin [5, 9]. It is also expressed in the prostate, and undergoes intracellular relocalization in prostate cancer compared with normal epithelia [10]. Derry et al. demonstrated that PTK6 is localized to nuclei of luminal epithelial cells in the normal prostate. However, loss of PTK6 nuclear localization occurs in prostate cancer.

In well differentiated tumors PTK6 maintained some nuclear localization, while in poorly differentiated tumors, complete loss of nuclear PTK6 was observed [10]. In cancer cells, the active pool of PTK6 is at the plasma membrane and this can be detected by examining phosphorylation of PTK6 tyrosine residue 342 with phospho-specific antibodies [11-13]. Targeting PTK6 to the nucleus in prostate cancer cells reduced growth [14], while targeting active PTK6 to the plasma membrane is oncogenic and was sufficient to transform mouse embryonic fibroblasts lacking SRC-family kinases SRC, YES, and FYN [15]. Although it is clear that PTK6 shuttles out of the nucleus in prostate tumors, further study is required to understand how it is relocalized and activated at the plasma membrane in prostate disease [16].

Analysis of publicly available datasets shows an increase in *PTK6* gene expression in advanced prostate cancers (Figure 1). Alterations in the *PTK6* gene are also found in prostate cancer and are summarized in Figure 2

PTK6 in prostate cancer

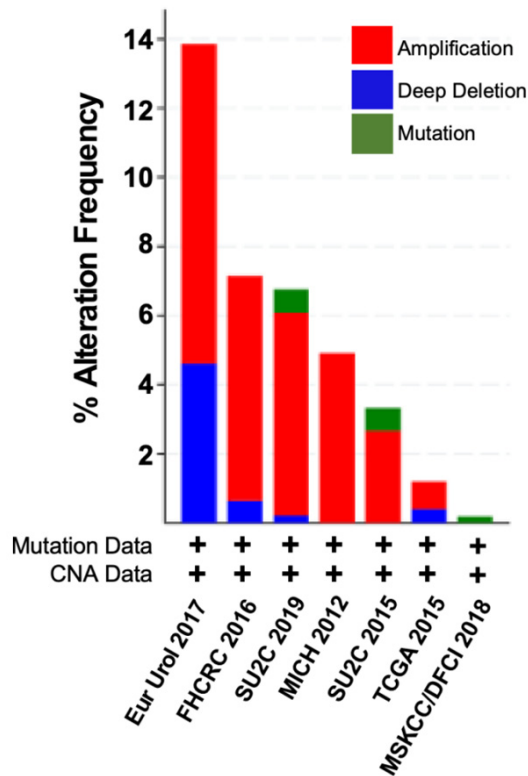


Figure 2. Alterations in the *PTK6* gene in prostate cancer. Analysis of the cBioPortal database (<https://www.cbioportal.org/>) indicates that gene alterations in *PTK6* occur in prostate cancer. These include both copy number alterations (CNA) and mutations. These datasets correspond to those in **Table 1** but differ in number because they represent the percentage of total samples, while **Table 1** expresses percentages over total patient number queried for *PTK6* gene alterations. Mutations identified include a missense mutation within the kinase domain V315M, and a truncating deletion mutant.

and **Table 1**. In addition, a variant of *PTK6* is encoded by an alternatively spliced *PTK6* transcript [17] that has been characterized in prostate cancer cells [18]. The alternative transcript encodes a 134 amino acid protein, ALT-*PTK6*, that shares the first 77 amino acids of the full length *PTK6*, including the SH3 domain. ALT-*PTK6* can interact with other proteins through its SH3 domain and competes with full length *PTK6* for SH3 binding sites. It may also be capable of binding SH3 domains of other proteins through its proline rich C-terminus. Overexpression of ALT-*PTK6* promoted nuclear translocation of full length wild type *PTK6*, leading to inhibition of beta-catenin and Sam68 [18]. ALT-*PTK6* also blocked phosphorylation of the *PTK6* substrate p27 (Kip1) and inhibited its ability to act as an assembly factor for CDK/Cyclin complexes [19].

Increased *PTK6* expression may occur due to gene amplification. In 202 breast cancers tested, *PTK6* was amplified in 57 samples, and co-amplified with *ERBB2* in 28 samples [20]. Irie and colleagues discovered amplification of *PTK6* in 15 out of 93 breast cancer patient samples [21]. Several public datasets indicate that amplification of the *PTK6* gene also occurs in prostate tumors (**Figure 2**). Genomic copy number alterations have been correlated with prostate cancer relapse [22]. Regulation of *PTK6* transcription in the prostate is not well understood. The ribosomal S6 kinase RSK was shown to upregulate *PTK6* RNA expression in prostate cancer cells [23].

Several miRNAs have been discovered to regulate *PTK6* in cancer, including miR-93, miR187, miR-17 and miRNA-214 [24-27]. Overexpression of miRNA-214 in prostate cancer cell lines hindered tumor cells growth, colony forming ability, invasion and migration. The effect of miR-214 on tumorigenesis was attenuated by overexpression of *PTK6*. Furthermore, miR-214 was discovered to target the 3'UTR-region of *PTK6* and subsequently regulates *PTK6* expression. Further experiments showed that miR-214 cooperates with ibrutinib to further inhibit *PTK6* activity and trigger prostate cancer cell death [25].

PTK6 activity is negatively regulated by phosphatases, including Protein Tyrosine Phosphatase 1B (PTP1B) [28] and the Phosphatase and Tensin Homologue (PTEN) tumor suppressor protein [13]. Both of these phosphatases dephosphorylate *PTK6* at tyrosine residue 342, thereby inhibiting its activity. Introduction of PTEN into *PTEN* null prostate cancer cells led to decreased *PTK6* activity coupled with reduced activation of its substrates FAK, BCAR1 (p130CAS) and AKT [13].

PTK6 contributions to oncogenic signaling in prostate cancer

PTK6 is activated by receptor tyrosine kinases including EGFR [29], IGF1R [21], and MET [30], all of which play roles in advanced prostate cancer [31-33]. In turn, *PTK6* activates several downstream oncogenic pathways. It phosphorylates the scaffolding protein BCAR1, leading to enhanced cell migration [11]. *PTK6* also directly phosphorylates FAK, both at the activation site (Y576/577) and at the GRB2 binding site (Y925) [15]. *PTK6* also

PTK6 in prostate cancer

Table 1. *PTK6* gene alterations and mRNA expression

Prostate Cancer Dataset	# Patients	% <i>PTK6</i> Gene Alterations	% High <i>PTK6</i> mRNA	<i>PTEN</i> Deletion/ Mutation	<i>TP53</i> Deletion/ Mutation	Reference
MICH, 2012	59	3/59 (5%)	NA	51%	51%	[50]
SU2C, 2019	429	30/429 (7%)	11/429 (3%)	34%	41%	[51]
SU2C, 2015	150	5/150 (3%)	6/118 5%	39%	51%	[52]
FHCRC, 2016	54	9/54 (17%)	6/63 (10%)	44%	33%	[53]
Eur Urol, 2017	65	9/65 (14%)	NA	11%	6%	[54]
TCGA, 2015	333	7/333 (2%)	21/290 7%	17%	8%	[55]
MSKCC/DFCI, 2018	1013	2/1013 (<0.2%)	NA	16%	21%	[56]

Publicly available datasets from cBioPortal (<https://www.cbioportal.org/>) were examined for *PTK6* gene alterations and expression levels. Percentages of *PTK6* gene alterations, high mRNA expression, as well as *PTEN* and *TP53* deletions and mutations were determined based on the total number of patients queried in each dataset.

phosphorylates EGFR at Y845, stimulating its activity and inhibiting its turnover [34].

PTK6 plays critical roles in regulating cell survival [15, 21, 23, 35-37]. Though not essential, *PTK6*-mediated activation of FAK promotes survival signaling and protects cells from anoikis [15]. *PTK6* directly phosphorylates AKT on tyrosine residues 315 and 326 and promotes its activation by EGF in prostate cells [38]. Survival signaling induced by *PTK6* is dependent on AKT, suggesting that AKT is a critical player downstream of *PTK6* [15].

PTK6 was targeted to the plasma membrane by the addition of a palmitoylation/myristoylation signal at the amino terminus of the kinase. This led to a dramatic change in cell morphology, formation of peripheral adhesion complexes and migration, dependent on BCAR1. *PTK6* phosphorylates BCAR1 and subsequently activates ERK5, suggesting that *PTK6* activation at the membrane is indispensable for ERK5 and BCAR1 activity [11].

Activation of *PTK6* at the plasma membrane was subsequently shown to promote the epithelial mesenchymal transition (EMT) [12]. The EMT program is associated with characteristics of metastatic cancer, cancer stem cells, chemotherapy resistance, and immune evasion [39, 40]. Ectopic expression of membrane targeted *PTK6* in PC-3 and BPH-1 prostate cells induced a mesenchymal like phenotypic change, which was coupled with a decrease in E-cadherin protein levels and increased expression of the EMT markers vimentin, ZEB1 and SLUG. These cells were more tumorigenic and metastatic in immunocompromised mice. Correspondingly, PC3 cells with knockdown of

PTK6 exhibited reduced survival and metastasis in a xenograft model [12].

The therapeutic potential of *PTK6* in prostate cancer

Targeting *PTK6* has shown potential for treating prostate cancer in animal models. Disruption of the mouse *Ptk6* gene in mice with conditional disruption of *Pten* in the prostate impaired prostate tumorigenesis [13]. *PTEN* loss is common in castration resistant prostate cancer [41], and there was a significant correlation between *PTK6* activation at the plasma membrane and loss of *PTEN* expression in human prostate tumor tissue microarrays [13].

Although *PTK6* is not currently targeted in prostate cancer patients, progress has been made in identifying compounds that target *PTK6* activity [42-44]. Vemurafenib or PLX4032, is an inhibitor of mutant BRAF^{V600E} in metastatic melanoma, but it can also target *PTK6* kinase activity with surprising specificity. Treatment of prostate cancer cells with vemurafenib reduced EGFR, FAK, AKT and ERK1/2 activation promoted by *PTK6*. In preclinical studies, administration of the vemurafenib ortholog used in mice PLX4720, decreased growth of xenograft prostate tumors compared with untreated controls [42].

Since *PTK6* activity is negatively regulated by the tumor suppressor *PTEN*, *PTK6* inhibitors may have particular efficacy in *PTEN* null prostate cancers. *PTEN* is mutated or deleted in about 25% of primary prostate tumors [45], and loss of *PTEN* or activation of PI3K is observed in up to 70% of advanced human prostate cancers [46]. *PTEN* loss has been shown to accel-

erate prostate tumorigenesis in vivo [47], and is associated with CRPC and lethal disease [48, 49]. Advances in understanding the roles of PTK6 in tumorigenesis and oncogenic signaling suggest that it may be a promising therapeutic target.

Conclusions

Although most studies have focused on the role of PTK6 in breast cancer, an indispensable role has been identified for PTK6 in prostate cancer signaling (reviewed here and in [16]). PTK6 has been shown to be important for prostate tumor growth, invasion and metastasis in animal models [12, 13, 42]. Activation of an EMT program downstream of PTK6 may represent a mechanism leading to resistance to monotherapies and increased cancer cell survival. Targeting PTK6 may have particular value in prostate cancers that have lost PTEN and subsequently have increased PTK6 activity. Several PTK6 associated kinases may be valuable therapeutic targets in prostate cancer including EGFR/EBB2, MET, IGF1R, and PI3K, and combinatorial therapies targeting them and PTK6 may have unique therapeutic advantages.

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Disclosure of conflict of interest

None.

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PTK6 in prostate cancer

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PTK6 in prostate cancer

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PTK6 in prostate cancer

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