PROTEIN TYROSINE KINASE-6 (PTK6)

Amanda Harvey

Institute for Environment Health and Societies

Brunel University London

United Kingdom

amanda.harvey@brunel.ac.uk

Synonyms

Breast Tumor **K**inase (Brk)

Historical Background

The intracellular protein tyrosine kinase, PTK6 (also known as the breast tumor kinase, Brk), has been implicated in the development and progression of a number of different tumor types. First identified in 3 separate studies in the early 1990s, it was initially found in a study to determine which tyrosine kinases were expressed in human melanocytes and then the full-length sequence was subsequently cloned from metastatic breast cancer using a screen to identify novel kinases that were expressed in tumors, but not normal breast tissue. Identification of the murine orthologue, sik, in mouse intestinal cells was achieved by the generation of a library of kinase catalytic domains.

PTK6 is related to the Src family of protein kinases and belongs to a distinct class of enzymes which includes Frk and SRMS (reviewed Harvey and Burmi 2011) and expression produces two different isoforms as a result of alternative splicing (reviewed in Hussain and Harvey 2014) (Figure 1).

Figure 1 — PTK6 Figure 1.tif

Figure 1: PTK6 domain structure. Schematic representation of the PTK6 gene structure and its products. All 8 exons are contained in the PTK6 transcript, resulting in production of a full length, fully functional protein. ALT-PTK6 arises as a result of alternative splicing, excluding exon 2. The subsequent protein shares the amino-terminus and the SH3 domain with its full length isoform and a frameshift results in the carboxy-terminus being truncated and proline rich (PR)

Under normal physiological conditions, PTK6 expression is highly controlled and is usually limited to differentiating cells of epithelial origin. Several studies have reported that, pathologically (i.e. in disease tissue), PTK6 expression is increased in a number of different cancers compared to normal cells of the same tissue type (reviewed in Harvey and Burmi 2011).

The apparent disparity in PTK6 expression and the difference in proliferation status between cellular differentiation in normal tissues, where cells tend not be dividing; and the fact that tumors are highly proliferative (and cells are dividing rapidly) makes PTK6 an intriguing molecule for study, as there are likely to be both context and function-specific differences that could be exploited in the development of anti-tumor therapies which conferring benefit to patients.

Cellular Localization

PTK6 has been reported to have different functions in different tissue types; for example, in normal tissues PTK6's role appears to be related to regulating the differentiation process, whereas in tumors PTK6 promotes proliferation and cell survival. Variations in cellular localization are thought to be associated with PTK6's opposing roles in both differentiation and proliferation. Altered cellular localization will no doubt affect the variety of substrates and binding partners that are available for PTK6, thereby contributing to the different functions/effects that have been ascribed to PTK6 expression.

Myristoylation is a post- or co-translational protein modification, whereby a fatty acid-derived group is attached to an N-terminal amino acid. Such modifications allow proteins to associate with membrane structures. Although PTK6 is structurally related to Src, it lacks the amino-terminal myristoylation site (Mitchell et al. 1994). Without a myristoylation site, PTK6 is not able to associate directly with the plasma membrane, and therefore cellular localization is not tightly regulated. Originally PTK6 was thought to be solely a cytoplasmic kinase, however, it is now known to be in different cellular compartments, including at the membrane via association with its binding partners as well as in the nucleus. Association of a protein to the plasma membrane can be mimicked by experimental inclusion of a myristoylation site. Adding a myristoylation site to the N-terminus, enhanced PTK6's oncogenic role by promoting cell proliferation, survival and migration of human embryonic kidney cells. Trapping PTK6 in the nucleus with synthetic nuclear localization signal abrogated these effects (Kim and Lee 2009); these *in vitro* experimental effects are supported by evidence from human tumors demonstrating that normal prostate epithelial cells and well-differentiated prostate carcinomas had nuclear PTK6, whereas as poorly differentiated prostate cancers were found to have cytoplasmic PTK6 (reviewed in Goel and Lukong 2015). Increased expression of ALT-PTK6 resulted in an increase of constitutively active PTK6 in the nuclei of prostate tumor cells, suppressing proliferation (Brauer et al. 2011). Collectively, these studies suggest that PTK6's oncogenic role may be dependent on its cellular localization; however, .as PTK6 contains neither myristoylation sites nor nuclear localization signals, it is unclear how PTK6 delocalizes

from one sub-cellular compartment to another and precisely which cellular signals are controlling this transition.

PTK6 Substrates and Binding Partners

As a tyrosine kinase with amino acid sequence homology to Src, PTK6 also has a similar domain structure consisting of SH2 and SH3 domains (which typically interact with phosphorylated tyrosines and proline-rich sequences respectively) as well as a kinase domain (which phosphorylates its target substrates on tyrosine). It is therefore capable of phosphorylating a number of target molecules and there is a rapidly expanding list of known interacting proteins (summarized in Table 1). To date, over 35 PTK6-associated interactions have been identified (reviewed in Goel and Lukong 2015), but whether all of these interactions result in phosphorylation has not yet been determined. PTK6 is proposed to have some kinase independent function (reviewed Harvey and Burmi 2011)), suggesting that a functional kinase domain is not required for all of PTK6's activities. It has been suggested that it may function as an adaptor molecule, and therefore it is highly likely that not all the interacting proteins will be substrates of PTK6's kinase activity. Therefore it is possible that one of PTK6's functions is to stabilize signaling complexes to allow phosphorylation of some of its interacting proteins and/or additional molecules within the complex by other kinases. This association in a large signaling complex, as an adaptor or scaffolding molecule, may also contribute to PTK6's cellular localization.

Localization of PTK6-interacting proteins		
Membrane Proteins	Intracellular Cytoplasmic Proteins	Nuclear-Protein
EGFR	ARAP-1	β-Catenin*
HER2	IRS-1	Sam68
ErbB3	IRS-4	SLM-1
ErbB4	Erk5	SLM-2
IGF-1R	β-Catenin*	PSF
ADAM-15A	Akt	KAP3A*
ADAM-15B	Erk	STAT3*
β-Catenin*	MAPK	STAT5a*
c-Cbl*	PTEN	STAT5b*
FAK*	Paxillin*	Dok1*
Paxillin*	KAP3A*	Hsp90*
p130CAS*	STAT3*	Hsp70*

β-Tubulin*	STAT5a* STAT5b* STAP-2 (BKS) GNAS FL139441 GapA-p65 C-Cbl* Dok1* FAK* Hsp90* Hsp70* p190RhoGAP p130CAS* β-Tubulin*	p27Kip1

Table 1: Summary of the localization of the major identified PTK6-interacting proteins (see Goel and Lukong 2015 for a comprehensive review of the biological outputs of these interactions) . A further two substrates have been reported; however the proteins involved (which are 23 kDa and 100 kDa in size) have yet to be identified.

* These proteins are reported to have functions in different cellular compartments, however it has not yet been determined whether PTK6 affects the function of these proteins in all their localizations.

PTK6 and Signaling Pathways

From the wide variety of substrates and interacting proteins that have already been identified (Table 1), it is clear that PTK6 plays a role in a number of different cellular processes and signaling pathways (Figure 1). This is also apparent from the range of signaling molecules that activate PTK6, which include epidermal growth factor (EGF), Heregulin (HRG), insulin-like growth factor (IGF), Hepatocyte growth factor (HGF), osteopontin (OPN) and calcium ions.

PTK6 has been shown to associate with all members of the ErbB receptor family (reviewed in Hussain and Harvey 2014) and potentiates the proliferative effects of EGF by activating the phosphoinositide 3-kinase (PI3-K)/Akt signaling pathway. PTK6 is known to play a role in cell migration through the phosphorylation of paxillin, which is a ‘molecular scaffold’ that interacts with signaling molecules and a

number of proteins that are involved in cell motility. In response to EGF, activated PTK6 phosphorylates paxillin resulting in the activation of Rac1 and an increase in cell migration. Treatment of breast cancer cells with the ErbB ligand HRG also increased cell migration, via activation of both Erk5 and an Erb-PTK6-Rac-p38 MAPK signaling pathway ((reviewed in Goel and Lukong 2015; Ostrander et al. 2007).

PTK6's interactions with the nuclear STAR (Signal Transduction and Activation of RNA) proteins (Sam68 and SLMs) and polypyrimidine tract-binding protein-associated splicing factor (PSF) are induced in response to EGF treatment. Phosphorylation of PSF and Sam68 (as well as the Sam68-like mammalian proteins, SLM1 and SLM2) by PTK6, results in inhibition of their RNA-binding activities. Reducing their RNA binding capability is one mechanism of reducing the function of proteins that bind RNA. As these proteins regulate a number of RNA processing events, including alternative splicing, PTK6 activation could result in the post-transcriptional regulation of gene expression. In addition EGF-mediated activation of PTK6 induced phosphorylation of Sam68 which increased cell proliferation. This presumably occurred through suppressing the anti-proliferative properties of Sam68 (Lukong et al, 2005; reviewed in Harvey and Burmi 2011).

PTK6 expression has also been linked to the potential regulation of IGF signaling. PTK6 interacts with the IGF-1R/IRS-1 complex resulting in increased activation of IGF-1R, as well as Akt. An increase in IGF-1-mediated, anchorage-independent cell survival was also observed in both breast and ovarian cancer cells (Irie et al. 2010). Similar effects on cell survival have also been shown in serum stimulated breast cancer cells, where PTK6 again protected against cell death in suspension culture (Harvey et al. 2009). Interestingly, in both IGF-1 stimulated cells and lapatinib-resistant cells PTK6 protected cells from classical apoptosis/anoikis (Irie et al 2010; Park et al 2015), whereas the Harvey study showed that PTK6 protected breast cancer cells from cell death via autophagy. Taken together, these studies indicate that PTK6 can protect cells from different types of programmed cell death, and that this protection is through different mechanisms.

Furthermore, treatment of breast cancer cells with the Met receptor ligand, HGF, induced both activation of Erk5 and cell migration (Castro et al. 2010). The PTK6/Erk5 interaction and the increased migration that was induced in response to HGF was not dependent on the kinase activity of PTK6, providing further evidence that some aspects of PTK6 function are not reliant on its kinase domain. This implies that PTK6 could coordinate the large signaling complexes that are required for cell migration, without directly phosphorylating components of the complex.

Most of the PTK6-mediated interactions and activation events result in altered cell behaviors such as increased proliferation, cell survival, migration and the secretion of angiogenic factors, all of which are traits that are characteristic of tumor cells and are required for tumor development (Reviewed in Hanahan and Weinberg 2000). It would appear from the majority of studies carried out in tumor cell lines, that PTK6 could play a central role in tumor progression, especially in breast cancer. In support of this hypothesis Chakraborty and colleagues showed that treating cells with OPN resulted in PTK6 activation and an increase in vascular endothelial growth factor (VEGF) production. Combined with *in vivo* models, they were able to demonstrate that OPN triggers VEGF-dependent angiogenesis (formation of new blood vessels) and tumor growth as a result of PTK6 activation (Chakraborty et al. 2008).

In cells where PTK6 is physiologically expressed i.e normal differentiating cells, the effects of PTK6 activation are distinct to those seen in tumor cells. When keratinocytes (skin cells) are treated with calcium, PTK6 is transiently activated and cell differentiation is induced, in contrast to the proliferation or migration seen in tumor cells. Recent evidence suggests that PTK6, the EGF receptor (EGFR) and a marker of differentiation (involucrin), may be co-regulated during differentiation, and that the differentiation of normal primary human keratinocytes could be influenced by altered PTK6 expression (reviewed in Harvey and Burmi 2011; Goel and Lukong 2015).

It is clear that PTK6 signaling is diverse (Figure 2), and that PTK6's function with respect to signaling is different in normal differentiating cells to that in tumor progression. PTK6 has also been shown to

associate directly with Akt (Zhang et al 2005) and, in conjunction with results from several other studies, has lead to the hypothesis that one of PTK6's roles in normal cells could be to constrain Akt activation, possibly to allow differentiation to occur. In tumor cells, the constraint is lost and the PI3-K/Akt pathway subsequently becomes activated, thereby up-regulating the processes that are known to be involved in tumor growth.

Figure 2 — PTK6 Figure 2.tif

Figure 2: Potential biological effects of PTK6-mediated interactions.

PTK6 Expression Profile

PTK6 expression is increased in many tumors compared to normal epithelial cells (reviewed Harvey and Burmi 2011) and this is most notable and most well characterized in breast tissue. It is only recently that PTK6 expression was detected in normal breast cells (Peng et al. 2014), with previous studies demonstrating that up to 86% of breast cancers have elevated PTK6 expression and that the increase in expression correlated with an increase in tumor grade (reviewed Harvey and Burmi 2011). is mediated by several factors including NFκB, Sp1, HIF1α, and the glucocorticoid receptor and correlates with HER2 overexpression and/or amplification in some tumors (reviewed in Goel and Lukong 2015;). This is important from a tumor development stand-point, as interaction of PTK6 protein with HER2 induces and prolongs the activation of the MAPK pathway which would result in increased cell cycle progression and tumor cell proliferation and, in mouse models, PTK6 expression promotes ErbB2-induced tumorigenesis (Peng et al. 2015).

Whilst not the whole picture, and the clinical significance is currently far from clear, a number of studies have also reported molecular changes that could lead to elevated PTK6 expression (reviewed Harvey and Burmi 2011), regulation by microRNAs (Haines et al. 2016), as well as missense and truncating mutations that could affect PTK6 function (reviewed Goel and Lukong 2015). This suggests that there are multiple mechanisms contributing to PTK6's overexpression.

These findings, taken together alongside the current knowledge of PTK6's multiple signaling roles in tumor cells, suggest that PTK6 is strongly implicated in tumor development. It could therefore be expected that PTK6 expression would have a negative impact on breast cancer patient survival and Kaplan-Meier survival analysis supports this conclusion (Pires et al. 2014).

Summary

There is little doubt that the role of PTK6 is complex. It depends on a number of factors including cellular localization, accessibility of binding partners, availability of extracellular signaling molecules and potentially, at least in tumor cells, the extent to which PTK6 expression is elevated. Further studies are fundamental to unraveling the complex and distinct roles that PTK6 plays in both normal differentiating cells and in tumor development. Progress in the isolation and development of compounds that inhibit PTK6's kinase activity, along with recent elucidation of the crystal structure, are fundamental to furthering our understanding of the distinctions between a pro-differentiation role in normal cells, and a potentially oncogenic role in tumor progression; this is key to the development of novel therapeutic agents that could target either context-specific or cell-specific molecular functions and/or interactions with minimal effects in other tissues.

See Also

Src

STAT3

MAPK

PI3K/Akt

EGFR

References

Brauer PM, Zheng Y, Evans MD, Dominguez-Brauer C, Peehl DM and Tyner AL. The alternative splice variant of protein tyrosine kinase 6 negatively regulates growth and enhances PTK6-mediated inhibitionof β-catenin. PLoS One. 2011; 6(3):e14789. doi: 10.1371/journal.pone.0014789

Castro NE and Lange CA. Breast tumor kinase and extracellular-signal-regulated kinase 5 mediate Met receptor signaling to cell migration in breast cancer cells. Breast Cancer Res. 2010;12(4):R60. doi:10.1186/bcr2622.

Chakraborty G, Jain S and Kundu GC. Osteopontin promotes vascular endothelial growth factor-dependent breast tumor growth and angiogenesis via autocrine and paracrine mechanisms. Cancer Res. 2008;68(1):152-61. doi: 10.1158/0008-5472.CAN-07-2126.

Goel RK and Lukong KE. Tracing the footprints of the breast cancer oncogene BRK – Past till present. Biochimica et Biophysica Acta. 2015;1856:39-54. doi: 10.1016/j.bbcan.2015.05.001

Hanahan D, and Weinberg RA. Hallmarks of Cancer. Cell 2000;100:57-70.

Haines RJ, Beard Jr RS, Eitner RA, Chen L and Wu MH. TNFα/IFNγ mediated intestinal epithelial barrier dysfunction is attenuated by microRNA-93 downregulation of PTK6 in mouse colonic epithelial cells. PLoS ONE. 2016; 1-18. doi:10.1371/journal.pone.0154351

Harvey AJ, Pennington C, Porter S, Burmi RS, Edwards DR, Court W et al. Brk protects breast cancer cells from autophagic cell death induced by loss of anchorage. Am J Pathol. 2009;175(3):1226-1234. doi: 10.2353/ajpath.2009.080811

Harvey AJ and Burmi RS. future therapeutic strategies: implications for Brk targeting. In: Gundez E and Gundez M, editors. InTech: Breast Cancer – Current and Alternative Therapeutic Modalities. 2011. p.413-435

Hussain HA and Harvey AJ. Evolution of breast cancer therapeutics: Breast tumour kinase's role in breast cnacer and hope for breast tumour kinase targeted therapy. World J Clin Oncol. 2014;5(3):299-310. doi: 10.5306/wjco.v5.i3.299

Irie HY, Shrestha Y, Selfors LM, Frye F, Iida N, Wang Z, et al. PTK6 regulates IGF-1-induced anchorage-independent survival. PLoS One. 2010;5(7):e11729. doi:10.1371/journal.pone.0011729.

Kamalati T, Jolin HE, Fry MJ and Crompton MR. Expression of the Brk tyrosine kinase in mammary epithelial cells enhances the coupling of EGF signaling to PI 3-kinase and Akt, via erbB3 phosphorylation. Oncogene. 2000;19(48):5471-6. doi: 10.1038/sj.onc.1203931

Lukong KE, Larocque D, Tyner AL and Richard S. Tyrosine phosphorylation of sam68 by breast tumor kinase regulates intranuclear localization and cell cycle progression. J Biol Chem 2005;280(46):38639-47. doi: 10.1074/jbc.M505802200

Ostrander JH, Daniel AR, Lofgren K, Kleer CG and Lange CJ. Breast tumor kinase (protein tyrosine kinase 6) regulates heregulin-induced activation of ERK5 and p38 MAP kinases in breast cancer cells. Cancer Res. 2007;67(9):4199-209. doi: 10.1158/0008-5472.CAN-06-3409

Park SH, Ito, K, Olcott W, Katsyv I, Halstead-Nussloch G and Irie HY. PTK6 inhibition promotes apoptosis of lapatinib-resistant HER2+ breast cancer cells by inducing Bim. Breast Cancer Research. 2015; 17:86 doi 10.1186/s13058-015-0594-z

Peng M, Emmadi R, Wang Z, Wiley EL, Gann PH, Khan SA, Banerji N, McDonald W, Asztalos S, Pham TN, Tonetti DA and Tyner AL. PTK^/BRK is expressed in the normal mammary gland and activated at the plasma memebrane in breast tumors. Oncotarget. 2014; 15(5):6038-6048. doi: 10.18632/oncotarget.2153

Peng M, Ball-Kell SM and Tyner AL. Protein tyrosine kinase 6 promotes ErbB2-induced mammary gland tumorigeneses in the mouse. Cell Death and Disease. 2015;6:e1848. doi:10.1038/cddis.2015.210

Pires IM, Blokland NJ, Broos AW, Poujade FA, Senra JM, Eccles SA, Span PN, Harvey AJ and Hammond EM. Hif-1α-independent hypoxia-induced rapid PTK6 stabilzation is associated with increased motility and invasion. Cancer Biol Ther. 2014; 15(10):1350-1357. doi: 10.4161/cbt.29822.

Regan Anderson TM, Ma SH, Raj GV, Cidlowski JA, Helle TM, Knutson TP, Krutilina RI, Seagroves TN and Lange CA. Breast tumour kinase (Brk/PTK6) is induced by HIF, glucocorticoid receptor, an dPELP1-mediated stress signaling in triple-negative breast cancer. Cancer Res. 2016; 76(6):1653-1663. doi: 10.1158/0008-5472.CAN-15-2510.

Zhang P, Ostrander JH, Faivre EJ, Olsen A, Fitzimmons D, Lange CA. Regulated association of protein kinase B/Akt with breast tumor kinase. J Biol Chem. 2005;280(3):1982-91. doi: 10.1074/jbc.M412038200

Figure 1

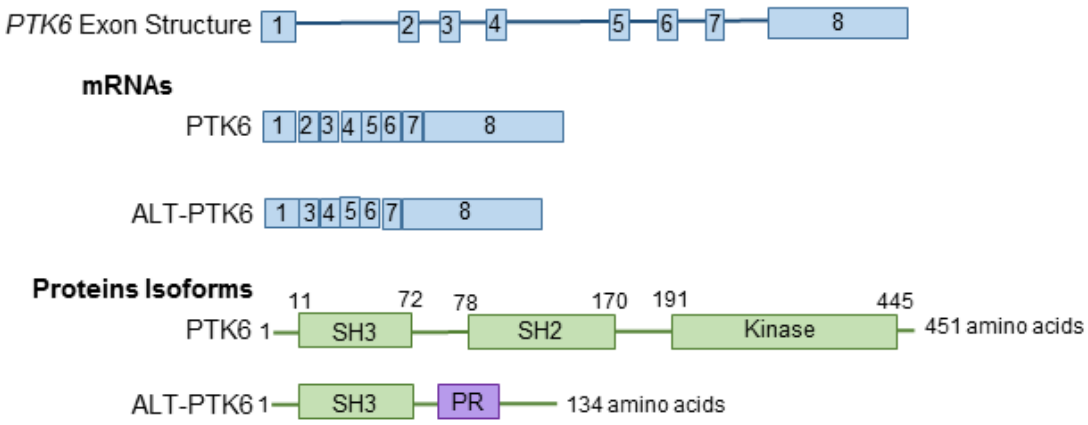
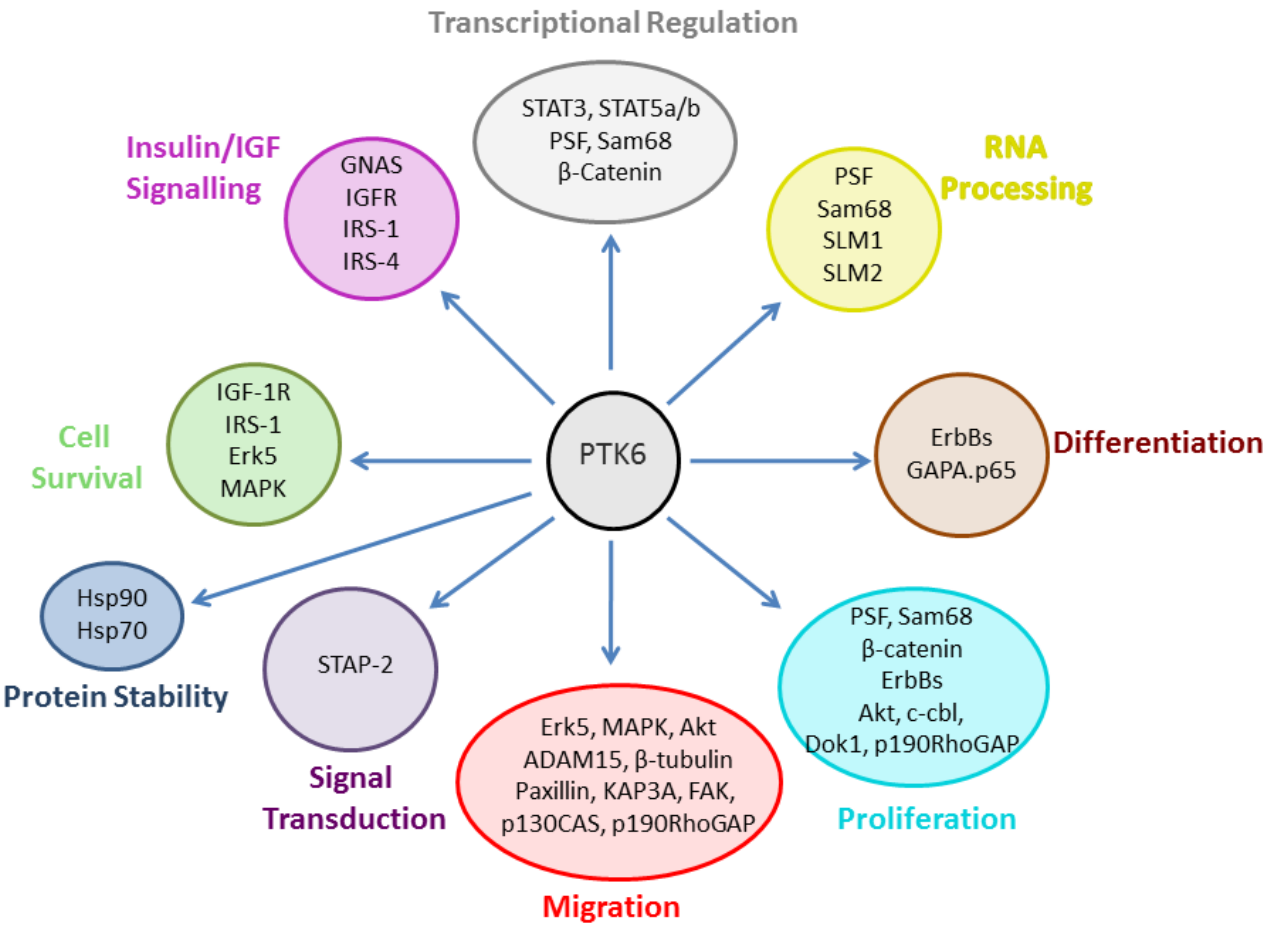

PTK6 Exon Structure
1
2
3
4
5
6
7
8
mRNAs
PTK6
1 2 3 4 5 6 7 8
ALT-PTK6
1 3 4 5 6 7 8
Proteins Isoforms
11
72
78
170
191
445
PTK6 1
SH3
SH2
Kinase
451 amino acids
ALT-PTK6 1
SH3
PR
134 amino acids

Figure 2


Transcriptional Regulation
STAT3, STAT5a/b
PSF, Sam68
β-Catenin
Insulin/IGF
Signalling
GNAS
IGFR
IRS-1
IRS-4
RNA
Processing
PSF
Sam68
SLM1
SLM2
IGF-1R
IRS-1
Erk5
MAPK
Cell
Survival
PTK6
ErbBs
GAPA.p65
Differentiation
Hsp90
Hsp70
Protein Stability
STAP-2
Signal
Transduction
Erk5, MAPK, Akt
ADAM15, β-tubulin
Paxillin, KAP3A, FAK,
p130CAS, p190RhoGAP
Migration
PSF, Sam68
β-catenin
ErbBs
Akt, c-cbl,
Dok1, p190RhoGAP
Proliferation