Review

BRK"ing" Down All We Know About PTK6 in Breast Cancer

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 doi:10.20944/preprints201808.0044.v1

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Abstract:

The search for improved therapeutic approaches to breast cancer are still on going. Breast tumor kinase (BRK, also known as PTK6) is one of the targets, it is highly expressed in breast carcinomas while displaying low or no expression in the normal mammary gland, which hints at the oncogenic role of this enzyme in breast cancer. In these twenty years, an increasing number of studies have focused on understanding the cellular roles of BRK in breast cancers. This review outlines the advances made towards understanding the cellular and physiological role of BRK, the molecular and chemical inhibitors and its therapeutic significance in breast cancer.

Keywords: Breast tumor kinase (BRK); Protein tyrosine kinase 6 (PTK6); Breast cancer; Pathways; Therapeutics

 doi:10.20944/preprints201808.0044.v1

1. Introduction

According to the American Cancer Society, breast cancer is the most common cancer among women. There are two main classification approaches for breast cancer. One is based on gene expression profile and the phenotype, where breast cancers are divided to 6 major subtypes: luminal A, luminal B, tumor enriched with human epidermal growth factor receptor 2 (Her2), basal-like, normal-like, and claudin-low subtype. The other method is based on hormone receptor expression, in which breast cancer can be divided into estrogen receptor (ER) and progesterone receptor (PR) positive, Her2 positive, and triple negative subtype [1]. So far, adjuvant endocrine therapy such as tamoxifen and aromatase inhibitors targeting ER signaling is used for the ER and PR positive subtypes, and the Her2 antibody Trastuzumab is used to treat the Her2 positive subtype, while the triple negative subtype, with none of the three markers, is associated with a poor prognosis.

Also known as protein tyrosine kinase 6 (PTK6), breast tumor kinase (BRK, pronounced "berk") was originally cloned from a metastatic human breast tumor in 1994 [2]. Since its discovery, there has been a growing number of publications on the expression levels and functions of this intracellular, non-receptor tyrosine kinase in different cell types. The BRK transcript is encoded by an 8.93kb length DNA located on chromosome 20q13.3 in humans, composed of 8 exons between 7 introns [3]. The protein is a 451-amino acid kinase, comprising of 3 parts, a classic Src homology 3 (SH3) domain and an Src homology 2 (SH2) domain, both of which are involved in protein-protein interactions, and a tyrosine kinase (SH1) domain [4] (Fig 1A). Compared to members of the Src family, BRK lacks the membrane anchoring N-terminal, which makes this protein soluble and accessible for interactions with intracellular substrates.

 doi:10.20944/preprints201808.0044.v1

Due to its ubiquitous nature, BRK was discovered to affect a large number of pathways, and most of the related published research has been on breast cancer. This review will hence focus on BRK in breast cancer, in an effort to consolidate what is currently known about this relatively novel, non-receptor protein tyrosine kinase.

BRK has contrasting functions in non-transformed and cancer cells [5]. BRK is highly expressed in in transformed cells of the breast, ovary, and metastatic melanoma cell lines, while its expression was low to undetectable in normal cells [2, 6]. BRK protein was found to be overexpressed in most breast cancer cell lines tested, about 85% of all breast carcinomas [7, 8] and its expression correlates with histological tumor grade, suggesting a possible oncogenic function for BRK. An alternative BRK transcript (ALT-PTK6) expressing just the SH3 domain has been discovered in breast cancer. The biological function of ALT-PTK6 is still unclear, however, it has been proposed that it may complete with wild type BRK for SH3 binding [9].

Besides overexpression, there is evidence that subcellular localization of BRK can contribute to its oncogenic function [7, 10]. BRK was found to be localized to the cytosol in breast cancers [10, 11]. Kim and Lee created constructs of BRK that localize either to the plasma membrane or the nucleus, and discovered that nucleus-targeted BRK had no oncogenic activity, compared to BRK targeted to the plasma membrane [12]. In 58 human prostate biopsy samples, it was reported that the location of BRK in the nucleus is related to the differentiation of prostate epithelial cells [13]. Plasma membrane-localized BRK was recently demonstrated to promote proliferation, migration and invasion through phosphorylation of Eps8, a protein involved in the EGFR pathway [14].

In addition to cellular localization, phosphorylation of BRK on tyrosine-342 (Y342) leading to its activation also plays a role in oncogenictity. Peng *et al.* added an additional dimension to the

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understanding of BRK in cancer when they discovered that there is no such phosphorylation in normal mammary tissues, in contrast to breast cancer cells [8]. They also revealed that the low levels of BRK found in normal mammary tissues were inactive and nucleus-localized, whereas the BRK found in transformed cells was plasma membrane-localized and possessed the phosphorylated Y342 signature, indicating the active form. While phosphorylation at site Y-447 stimulates its binding to the SH2 domain and negatively regulates kinase activity.

2. BRK signaling

BRK is involved in a number of pathways that play an important role in different cellular functions, as shown in Fig 1B.

2.1 ErbB/HER family pathway

The most well-known pathway associated with BRK is the ErbB (epidermal growth factor receptor-related receptor) family pathway. The first report to demonstrate the existence of an interaction between BRK and EGFR (epidermal growth factor receptor), has also showed that BRK expression could increase the proliferative activity of mammary epithelial cells [15]. Subsequently, Kamalati et al. then demonstrated that BRK, in response to EGF, phosphorylated erbB3 to result in recruitment of phosphoinositide 3-kinase (PI3K) and promotion of Akt signaling [15]. Through this, it was deduced that breast cancer cells expressing or overexpressing BRK would have a proliferative advantage. Other than EGFR and erbB3, studies have also shown associations of BRK with erbB2 [16], where there is co-amplification and co-expression of both proteins in breast cancer cells. BRK overexpression was found to selectively heighten the Ras/MAPK signaling pathway over the PI3K/Akt pathway through sustaining Erk1/2 (extracellular regulated kinase) activation.

 doi:10.20944/preprints201808.0044.v1

Additionally, BRK overexpression promotes erbB2-induced cell proliferation via increasing activation of the cyclinE-cdk2 complex [16]. ErbB2 has no identified endogenous ligand but heterodimerizes with erbB3 and 4, both of which bind heregulin. Heregulin was subsequently found to activate the tyrosine kinase activity of BRK [17], which resulted in activation of p38 MAPK and Erk5.

2.2 Akt pathway

From the above, it is apparent that there is a degree of uncertainty over BRK's effect on the PI3K/Akt pathway. Further studies conclude that BRK may limit Akt activity in normal cells but not in transformed cells, allowing BRK to potentiate the effects of growth factors [17]. It had been recently proven that there is indeed interaction between BRK and Akt in breast cancer cells, where this complex does not dissociate in response to EGF signaling, unlike that seen in normal cells [17]. By coupling the finding that wild-type (WT) BRK inhibited Akt activity, with the discovery that the BRK-Akt complex remained active in T47D cells, it had been speculated that BRK might be constitutively active in transformed cells [17]. In contrast to the above finding, another laboratory had found that BRK directly phosphorylates Akt on tyrosines 315 and 326 to activate the latter [18]. However, this was only concluded from transfecting constitutively active BRK (PTK6-Y447F) into HEK-293 kidney and SYF fibroblast cells, so it remains to be seen if this proves true in transformed cells.

2.3 Paxillin pathway

The EGF pathway also stimulates BRK's phosphorylation of the extracellular matrix tethering protein paxillin to promote migratory and invasive characteristics in breast cancer cells. BRK has been reported to directly phosphorylate paxillin at tyrosines-31/118 and promote migration via

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activation of Rac1 GTPase [19]. In a follow-up study, the research group managed to identify BRK's role in phosphorylating p190RhoGAP-A (p190-A) at tyrosine-1105 upon EGF stimulation to complement the finding above [20]. Phosphorylated p190RhoGAP-A then associates with p120RasGAP (p120) to inhibit the latter's activity, consequently leading to inhibition of RhoA and activation of the Ras oncogene to promote migration and invasion [20]. This was confirmed in breast cancer cell lines by the observation that RhoA and Ras regulation was lost after severing the association between p190-A and p120 [20].

2.4 IGF and insulin receptor family pathway

Besides the erbB family, the IGFR (insulin-like growth factor receptor) family has also been implicated with BRK. IGF-1R had been previously proposed as a breast cancer marker as it is found on all breast cancer subtypes to be indicative of poor prognosis [21]. If this holds true for in vivo data as well, the involvement of BRK in the IGF pathway could be very significant indeed. Endogenous BRK had been proven to be expressed in conjunction with IRS-4 (insulin receptor substrate) in the MDA-MB-231 breast cancer cell line, and this interaction between the two proteins was increased by IGF stimulation [22]. This notion that BRK plays a role in the IGF signaling pathway was ascertained by another study, which found that down-regulation of BRK in MCF-7 breast cancer cells resulted in a decreased IGF-1R autophosphorylation status that was not due to a drop in IGF-1R levels [23]. This eventually led to decreases in the Erk and Akt signaling downstream of IGF-1R.

2.5 STAT pathway

An interesting convergent point for the involvement of BRK between the EGFR and IGFR pathway is that both of these receptors mediate STAT3 (signal transducers and activators of transcription) activation [24]. Consequently, it was perhaps not surprising that BRK was found to mediate STAT3

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155 activation. This modulation is executed through BRK's interaction with BKS (BRK substrate)/STAP-2
156 (signal transducing activator protein-2), which subsequently interacts with STAT3 [25, 26]. STAP-2
157 was one of the first substrates of BRK to be uncovered and it is phosphorylated on tyrosine-250 by
158 BRK [25]. BRK, STAP-2 or STAT3 knockdown all gave similar degrees of reduction in T47D breast
159 cancer cell proliferation [26]. Intriguingly, Liu et al. found out that STAT3 is directly phosphorylated
160 by BRK on tyrosine-705 in a dose-dependent manner [27]. Tyrosine-705 phosphorylation is notable
161 as this causes the activation of the transcription activation domain (TAD) in STAT3. As this was
162 performed by an in-vitro kinase assay using COS-1 fibroblast-like cells, further explorations into this
163 will be needed to ascertain if it holds true in breast cancer cells. A possible mechanism of action to
164 explain the findings gathered here so far is that BRK, STAP-2, and STAT3 form a complex in cells
165 where BRK directly phosphorylates both STAP-2 and STAT3. This results in the activation of
166 STAP-2, which binds to STAT3 to further enhance the transcriptional activity of STAT3 [28], thereby
167 possibly explaining the finding that BRK and wild type STAT3 have a synergistic relationship that
168 results in a ten-fold induction in gene expression in STAT3-/- murine fibroblasts [27]. Furthermore
169 the inhibitor of JAK-STAT signaling, SOCS3 (suppressor of cytokine signaling) also inhibits BRK.
170 Besides STAT3, STAT5b is another molecule that interacts with STAP-2. However, while STAT3
171 binds to STAP-2 through its C-terminal YXXQ motif, STAT5b and STAP-2 interact through their PH
172 (pleckstrin homology) and SH2-like (Src homology) domains [28]. BRK was found to mediate
173 STAT5b phosphorylation at tyrosine-699, the activating residue of STAT5b [29]. The same group of
174 researchers also showed that in breast cancer cell lines expressing BRK, siRNA-mediated
175 knockdown of BRK or STAT5b respectively reduced DNA synthesis but there was no further
176 decrease for the double knockdowns [29].

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178 **3. Function of BRK in breast cancer**

179 As previously mentioned, BRK is implicated in numerous pathways and this translates to a
180 broad impact on the phenotype of cells. Figure 1B provides the link between BRK's molecular targets
181 and their subsequent downstream phenotypes.

182 3.1 Cell proliferation

183 When BRK is downregulated by RNA interference, breast cancer cells show a significant inhibition
184 of proliferation [30]. By phosphorylating p190RhoGAP, Derry et al. reported that BRK regulates Rho
185 and Ras to promote breast carcinoma growth, migration, and invasion [13], while Xiang et al.
186 reported this protein induces cell proliferation by activation of Ras/MAPK signaling and cyclin
187 E/cdk2 activity [31]. BRK is also found to be a key mediator in hypoxia-induced breast cancer
188 progression [32].

189 3.2 Cell migration and invasion

190 In 2004, BRK was firstly shown to promote EGF-induced cell migration [33]. Chen et al. reported that
191 EGF stimulation activates the catalytic activity of BRK, which in turn phosphorylates paxillin to
192 promote the activation of small GTPase Rac1 via the function of CrkII. Though this pathway, BRK
193 translocates to membrane ruffles and colocalizes with paxillin during cell migration. Besides these
194 effectors, KAP3A, ERK5, and Met signaling have also reported as physiological substrates of BRK
195 during cell migration [34, 35].

196 3.3 Survival

197 Besides promoting cell proliferation and migration in breast cancer cells, BRK plays a role not only
198 as a marker for survival in breast cancer, but also as an enhancer of cell survival. Aubele's group

 doi:10.20944/preprints201808.0044.v1

199 demonstrated that PTK6 is a prognostic marker of metastases-free survival in breast cancer [36], and
200 is independent of the classical morphological and molecular markers of lymph node involvement,
201 tumor size, and HER2 status [37]. Meanwhile, Harvey et al. reported that BRK enhances breast
202 carcinoma cell survival when grown in suspension, which suggests BRK plays a role in supporting
203 breast cancer cell dissemination [38]. BRK has also been reported to cooperate with HER2 and Src to
204 regulate breast cancer cell survival [39].

205 3.4 Angiogenesis

206 BRK is also involved in angiogenesis in breast cancer [40]. In this report, the authors found that
207 osteopontin triggers vascular endothelial growth factor-dependent tumor progression and
208 angiogenesis by activating BRK/nuclear factor-inducing kinase/nuclear factor-kappaB/activating
209 transcription factor-4 signaling cascades through autocrine and paracrine mechanisms.

210 3.5 Deregulation of Cell cycle

211 Though its expression does not change substantially throughout the whole cell cycle, BRK
212 deregulates the cell cycle by downregulating the cell cycle inhibitor p27 by inhibiting the
213 transcription factor FOXO's nuclear localization, thereby antagonizing their transcriptional activity
214 [41].

215 3.6 Apoptosis

216 The effect of BRK in apoptosis in breast cancer has been proven in 2 reports, Irie's group found that
217 BRK downregulation induces apoptosis of breast cancer cells deprived of matrix attachment [42]. 5
218 years later, Park et al. also reported similar results in Lapatinib-resistant Her2 positive breast cancer
219 cells, and their data showed BRK inhibition promotes apoptosis by inducing Bim [43].

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4. Therapeutic significance

Considering the distinctively elevated levels of BRK in a high proportion of human breast carcinomas as compared to normal tissue, according to literature [7] and our data (Fig 2A), and the causal relationship established between BRK overexpression and its various oncogenic roles including promoting cell proliferation and migration, it is unsurprising that BRK has been considered a future therapeutic target for the development of novel treatments in breast cancer. Moreover, the overall survival and disease-free survival curve (Fig 2C) shows that patients with low BRK have better survival than those with high BRK expression, which also indicates that utilization of a BRK inhibitor might play a role in breast cancer therapy. We have classified the BRK inhibitors into two categories: biological and chemical inhibitors (Table 1).

4.1 Biological inhibitors

Biological inhibitors are cellular compounds that target BRK and/or its associated pathways, of which three have been elucidated.

The suppressor of cytokine signaling 3 (SOCS3) protein was observed to be a negative regulator of BRK [44]. Conventionally, SOCS3 has been studied as a feedback inhibitor regulating the JAK-STAT pathway through both ubiquitin mediated proteasome degradation and non-competitive inhibition. BRK, as an activator of STAT3, was also found to be the target of both such negative modulations, with the latter having a greater impact.

Interestingly, heat shock protein 90 (Hsp90) inhibitors, such as geldanamycin, can also be considered as a therapeutic drug to indirectly inhibit BRK [45]. Proteosomal degradation of BRK is ubiquitin mediated, and this process is impeded by increased protein stability rendered through BRK-Hsp90 interaction. Geldanamycin, which prevents this heteroprotein complex formation,

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reduces BRK levels in a time-dependent manner in breast cancer cell lines T-47D and BT-474 and decreases phosphorylation of BRK substrates, while not affecting endogenous Src levels.

Protein-tyrosine phosphatase 1B (PTP1B) is another enzymatic regulator of BRK. Conventionally studied as an inhibitor of the IGF-1 signaling pathway, PTP1B also directly dephosphorylates and thereby inhibits BRK [46]. The activity of these three molecules is intertwined as BRK activates the IGF-1 receptor, the downstream target of PTP1B. Enhanced expression of PTP1B was shown to be effective against ovarian cancer cell lines in attenuating proliferation and anchorage-independent survival.

4.2 Chemical inhibitors

Besides the above biological molecular inhibitors of BRK, an increasing number of chemical inhibitors have also been studied. Recently, triterpene sipholenols, isolated from the Red Sea sponge Callyspongia siphonella, and their semisynthetic derivatives have been found to have a targeted effect on inhibiting proliferation in multi-drug resistant (MDR) cancer cells, including the highly metastatic MDA-MB-231 breast cancer cell line [47]. Furthermore, several sipholenols are highly selective BRK inhibitors, acting by inhibiting BRK phosphorylation in a dose-dependent manner. The most potent triterpene sipholenol BRK inhibitors were identified to be 4β-O-benzyl sipholenol A and 4β-O-benzyl-19,20-anhydrosipholenol A [50]. In most cases, the effectiveness of analogues in inhibiting BRK phosphorylation paralleled their anti-migratory ability [48].

Oleanolic Acid [49], extracted from Terminalia bentzoe L. leaves, is another triterpene whose semisynthetic derivatives have been optimized in anti-migration, anti-proliferation, and anti-invasion effects on the breast cancer cell line MDA-MB-231, and was further shown to induce apoptosis in four breast cancer cell lines: MDA-MB-231, MCF-7, BT-474, and T-47D. These effects

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were proposed to be in part due to the derivatives' ability to inhibit phosphorylation of BRK, along with Paxillin and Rac1, and also in part due to upregulation of FASL, leading to activation of RIP, BID, and various caspases, and eventually to the proteolytic cleavage of PARP-1 [50].

Phenylmethylene hydantoins [51], isolated from marine sponge Hemimycale arabica, and their semisynthetic analogues were explored as therapeutics for the breast cancer cell line MDA-MB-231, with anti-proliferation and anti-migratory effects, while being non-toxic to normal mammary epithelial cells at the concentrations tested on the cell line MCF-10A. (Z)-5-((4'-Fluorobiphenyl-10-yl)methylene)imidazolidine-2,4-dione was the most active of this class of compounds, significantly decreasing phosphorylation of Brk, paxillin, and Rac1, with little effect on the total levels of these molecules, similar to the previously discussed chemical inhibitors.

4-anilino α-carbolines are another class of compounds that have been studied as BRK inhibitors, of which 4-(m-hydroxyaniline)-α-carboline was found to be the most potent [52]. It was predicted that this inhibition occurs through interaction with BRK's ATP-binding pocket. These compounds were successful in limiting proliferation of MCF7, HS-578/T, and BT-549 breast tumor cancer cell lines, with correlation observed between effectiveness of BRK inhibition and anti-proliferative effects of these compounds. Furthermore, these compounds were found to modestly induce cell death of non-adherent breast cancer cells [53].

Imidazo[1,2-a]pyrazin-8-amines have also been found to interact with BRK's ATP-binding pocket and thereby inhibit it [54]. Biochemical studies optimized a subclass of analogues to be extremely potent to BRK, along with 300-fold sensitivity over potentially implicated compounds Aurora B and Lck. Furthermore, pharmacokinetic testing found the compound to have an appealing overall DMPK profile.

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Lastly, (E)-5-(benzylideneamino)-1H-benzo[d]imidazol-2 (3H)-one derivatives showed effectiveness in inhibiting phosphorylation of PTK6, with at least 20-fold selectivity over similar non-receptor tyrosine kinases, Src, Fyn, Bmx, and EGFR [55]. Bromine along with methoxide or ethoxide substitution of the benzimidazoline ring created the most active compounds targeting BRK, which were non-toxic to normal human foreskin fibroblast at IC50 levels required to inhibit the hepatic cancer cell line HEK 293. Pyrazolopyrimidine PP1 and PP2 were found to inhibit the catalytic activity of PTK6 in vitro. The chemicals work through suppression of phosphorylation of PTK6 substrates in HEK 293 cells. The authors also showed that the chemical inhibited PTK6-dependent proliferation of T-47D breast cancer cells [56].

Lastly, XMU-MP-2 specifically inhibits the kinase activity of BRK and downstream signaling pathways, resulting in the blockade of proliferation of breast cancer cells [57]. XMU-MP-2 used in combination with HER2 and ER inhibitors was also shown to block breast cancer cells proliferation *in vivo* and *in vitro*.

As shown in Fig 2B, BRK is highly expressed in ER positive breast cancer compared to ER negative breast cancer tissue, the same as HER2, which suggesting a correlation between ER or HER2 and BRK. Additionally, the survival curve (Fig 2C) and the response to tamoxifen or doxorubicin and Cyclophosphamide (Fig 2D) also suggest the candidate characterization of BRK as a novel therapy target in breast cancer. Although there has been much progress to identify novel drugs targeting BRK, translational research in this area is still lacking, with most papers focused on biological and cellular level studies. As such, there is still much potential in the exploration of therapeutics against BRK.

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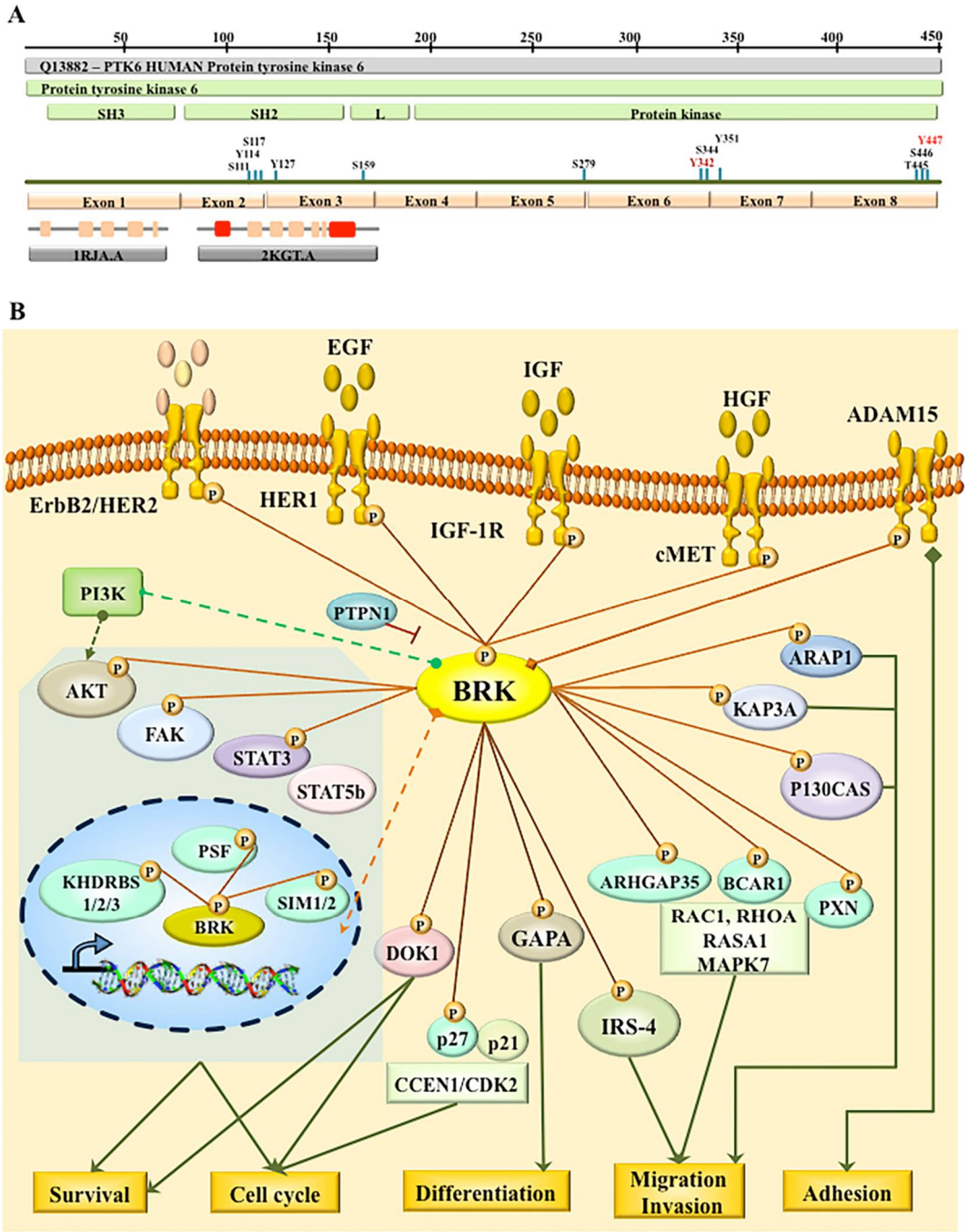

Figure 1. Structure, pathway and functions of BRK

A. The human *BRK gene* consists of 8 *exons* spliced between 7 *intros* regions. The DNA coding region spans 8.93 kb and the *mRNA* transcript is 2507bp long. The human BRK protein is a 451 amino acid kinase, which consists of 3 functional domains - SH3, SH2, and SH1 domain. The first two domains are required for interactions with other molecules, while the SH1 domain confers a catalytic role to the protein. Twelve modification sites have

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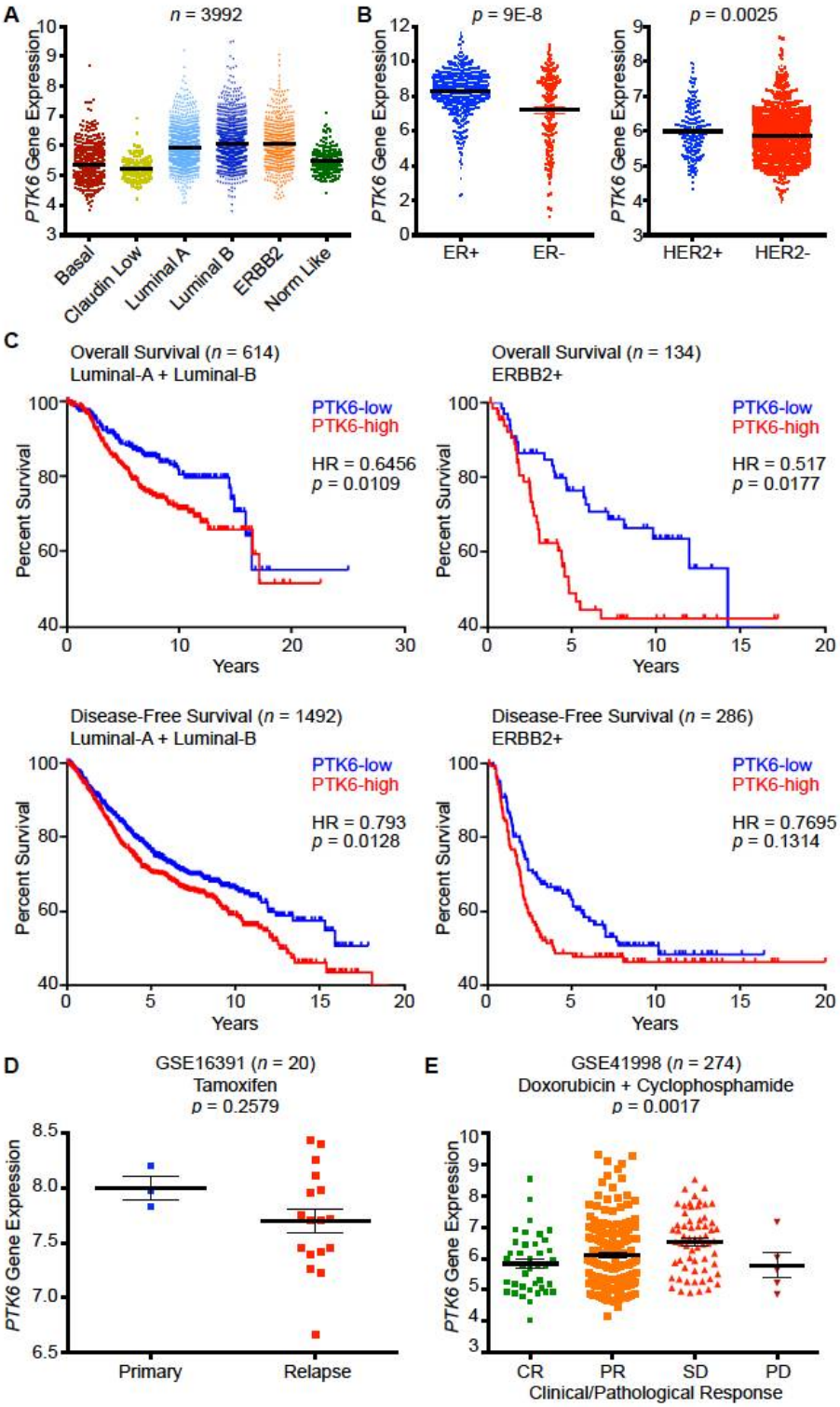
been reported in the human BRK protein and are indicated. **B.** BRK is implicated in the regulation of a variety of signaling pathways that control differentiation, cell survival, cell cycle progression, and cell motility, as well as tumor growth. BRK has been documented to interact with several substrates present in the nucleus and cytoplasm to drive growth of cells.



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319 **Figure 2.** Clinical characterization of BRK in breast cancer

320 **A.** BRK expression in breast cancer. **B.** BRK expression is significantly higher in ER + and HER2+ breast cancer

321 tissues compared to ER- and HER2- breast cancer tissues. **C.** The overall survival and disease-free survival in

322 BRK low Luminal-A and Luminal-B and ERBB2+ breast cancer is significantly higher than BRK high breast

323 cancer tissues. **D.** BRK expression in breast cancer tissue pre –versus-post tamoxifen treatment. **E.** BRK

324 expression in breast cancer tissue upon Doxorubicin and Cyclophosphamide treatment

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325 **Table 1.** Natural and chemical inhibitors of BRK

	Compound involved	Mechanism of action	Phenotypic effects	Cell type(s) used	References
Biological Inhibitors of BRK	**SOCS3**	- Associates with BRK through SOCS3 SH2 domain binding to BRK tyrosine kinase domain (KIR) - SOCS3 might induce BRK degradation with its E3 ubiquitin ligase binding domain as a secondary mechanism	- Attenuated proliferation	Breast cancer	[44]
	HSP90 inhibitors	- BRK-HSP90 interaction increase BRK stability - Geldanamycin, HSP90 inhibitors decreases phosphorylation of BRK substrates	- Attenuated proliferation	Breast cancer	[45]
	PTP1B	- Wild-type PTP1B dephosphorylates BRK at Y342, a site for tyrosine kinase activity - PTP1B dephosphorylates IGF-1β, a substrate of BRK which induces anchorage-independent cell survival	- Attenuated proliferation - Impaired anchorage-independent cell survival	Ovarian cancer	[46]
	Sipholenol A	- Inhibited BRK phosphorylation in a	-Potently inhibited migration at	Breast cancer	[47-48]

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	and Sipholenone A analogues (eg. sipholenol A 4β-4-chlorobenzoate and 19,20-anhydrosipholenol A 4β-4-chlorobenzoate esters)	dose-dependent manner, with no effect on total BRK binding affinity - Induced cell cycle arrest at the G1 phase - Might carry out its effects through interaction with FAK as well	approximately 5-6μM and invasion at 10μM - No cytotoxicity to normal cells at the respective concentrations above - Suppressed cell growth, migration and invasion		
	Oleanolic acid and analogues	- Potently targeted the BRK/Paxillin/Rac1 axis - Significant reduced p-Akt and pErk1/2 levels	- Most active analogue exhibited an IC_{50} value of 1.4μM in migration assay and 3.4μM in proliferation assay	Breast cancer Prostate cancer	[49,50]
Chemical Inhibitors of BRK	**Phenylmethylene hydantoins**	- Inhibited phosphorylation of BRK and Paxillin - Reduced c-MET and FAK expression levels	- Prevented tumor growth in mouse xenograph model - Non-toxic up to concentrations higher than their IC_{50} values in MCF10A cells - IC_{50} value of 3.8μM was achieved for the most active compound tested *in vitro* - Most active compound reduced migration	Breast cancer	[51]

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			in prostate cancer cells with an IC_{50} value of 15μM but was not very effective in breast cancer cells - No significant anti-invasive activity on both cancer cell types tested		
	4-anilino-α-carboline	- α-carboline ring makes hydrophobic interactions with residues within BRK's ATP-binding site	-Attenuated proliferation with GI_{50} value of 0.99μM - Induced cell death under loss of adherence conditions	Breast cancer	[52,53]
	Imidazo[1,2-*a*]pyrazin-8-amines	- Interact with BRK's ATP-binding pocket and thereby inhibit it	No function assay is performed		[54]
	(E)-5-(benzylideneamino)-1H-benzo[d]imidazol-2 (3H)-one derivatives	- Inhibit phosphorylation of PTK6	- Non-toxic to normal human foreskin fibroblast at IC50 levels required to inhibit the hepatic cancer cell line HEK 293.	Hepatic cancer	[55]
	Pyrazolopyrimidines PP1 and PP2	- Suppressed the phosphorylation of PTK6 substrate proteins, including signal transducer and activator of transcription 3	- Inhibited the PTK6-dependent proliferation of human breast carcinoma T-47D cells	Breast cancer	[56]
	XMU-MP-2	- Suppresses kinase activity of PTK6 and downstream signaling pathways	- Reducing proliferation	Breast cancer	[57]

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			in BRK-positive breast cancer cells. - Repressed the growth of tumors in mouse xenograft models driven by oncogenic BRK, - XMU-MP-2 cooperated strongly with HER2 inhibitor or ER blockade to block breast cancer cell proliferation in vitro and in vivo		

 doi:10.20944/preprints201808.0044.v1

5. Conclusions

In summary, we have introduced the background of BRK, and illustrated the molecular targets and pathways this enzyme interacts with, as well as the function of BRK in breast cancer and the involvement of its inhibitors in future targeted therapy for breast carcinoma. Over the 20 years since the discovery of this molecule, a rough picture for BRK has been painted, but there still are some questions about this protein that remain. Overall, a better understanding of the seemingly paradoxical functions of BRK in breast cancer would help in the development of novel drugs and targeted therapy for breast carcinoma and perhaps other cancers.

Funding: Grants from the National Medical Research Council of Singapore; the National Research Foundation Singapore and the Singapore Ministry of Education under its Research Centres of Excellence initiative to Cancer Science Institute of Singapore; National University of Singapore to Goh BC and Kumar AP. This work was also supported by grants from the NUHS Basic Seed Fund and Ministry of Education Tier 1 to Sethi G. The project was also supported by the Shenzhen Development and Reform Commission Subject Construction Project (2017)1434 to Lobie PE.

Author Contributions: Conceptualization: GS, PEL and APK.; Methodology: YY, HLA and XL; Software: TZT; Data Analysis: TZT.; Investigation: LW, VKP ; Resources: BBH, HYC, PZO, KSR, KST; Data Curation: YY and HLA; Writing-Original Draft Preparation: YY, HLA, XL, BBH, HYC, PZO, KSR, B; Writing-Review & Editing, BCG, RYH, FA, GS, PEL and APK; Supervision: BCG, RYH, GS, PEL and APK

Conflicts of Interest: The authors declare no conflict of interest.

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