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▮▮▮ REVIEW ARTICLE

The therapeutic efficacy for targeting the PI3K signaling pathway for fighting breast, prostate and multiple myeloma cancer cells

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▮▮▮ ABSTRACT

The PI3K signaling pathway is involved in the regulation of cancer cell growth, motility, survival and metabolism. This pathway is frequently active in many different types of cancer as breast, prostate and multiple myeloma. Targetable genetic aberrations in this pathway give the researchers many opportunities for the development of targeted therapies for different types of cancer. The high frequency of mutations in this pathway in multiple types of cancer has led to the development of small-molecule inhibitors of PI3K, several of which are currently in clinical trials. However, several feedback mechanisms either within the PI3K pathway or in compensatory pathways can render tumor cells resistant to therapy. Here, we give insight into the importance of the PI3K pathway as a target for cancer therapy and discuss the potential clinical efficacy of PI3K inhibitors. We mainly focused on the roles of PI3K signaling pathway in three cancer cell types including breast cancer, prostate cancer and multiple myeloma cancer.

BACKGROUND

The phosphoinositide 3-kinases (PI3Ks) are a family of lipid kinases that regulate intracellular signaling cascades in both normal physiology and disease (Cantley, 2002; Vivanco and Sawyers, 2002). PI3Ks are subdivided in three classes according to structure and substrate specificity (Vanhaesebroeck and Waterfield, 1999). In this context, mainly class IA PI3Ks has been implicated in the etiology of various diseases, including cancer. Class IA PI3Ks are heterodimers composed of a p110 catalytic subunit (α, β, and δ) and a p85/55 regulatory subunit that can be activated downstream of receptor tyrosine kinases (RTK), G protein–coupled receptors (GPCR), and small GTPases (Thorpe et al., 2015). Recently, PIK3R1 and PIK3CA, the genes encoding p85α and p110α, respectively, were found to be frequently mutated in several types of solid tumors which brought PI3Ks to be the forefront of cancer research (Philpet al., 2001; Samuels et al., 2004). The primary consequence of PI3K activation is the conversion of phosphatidylinositol-4,5-bisphosphate (PIP2) into the short lived second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3) on the inner leaflet of the plasma membrane. PIP3 recruits proteins to the membrane that contains a pleckstrin–homology domain, including AKT and its upstream activators PDK1 and mTORC2. At the

membrane, PDK1 phosphorylates AKT at T308 (Stokoe et al., 197) and mTORC2 phosphorylates it at S473 (Sarbassov et al., 2005), which results in full activation of the enzyme .

AKT acts as a major mediator of PI3K signaling by phosphorylating a wide range of substrates that regulate cell-cycle entry, survival, protein synthesis, RNA translation, glucose metabolism, and migration. PI3K activity is tightly controlled in cells and can be attenuated by lipid phosphatases, such as PTEN (stambolic et al., 1998), INPP4B (Gewinner et al., 2009) and SHIP2 (Clement et al., 2001) that dephosphorylate phospholipids in positions 3, 4, and 5, respectively, on the inositol ring. PIP3 is also an important signal upstream of several pro-oncogenic signals, including SGK3 (Vasudevan et al., 2009).
(Gasser et al., 2014) and PREX1/ PREX2 (Donald et al., 2004; Rosenfeldt, 2004). Over activation of the PI3K signal is one of the most frequent events in human cancers and can be achieved through alterations in most of the major nodes of the pathway (Thorpe et al., 2015). Activating mutations and/or amplification of the genes encoding the PI3K catalytic subunits p110a (Stokoe et al., 1997) and less frequently p110b (Gubens et al.,2016) mutations in the p85a regulatory subunit that abrogate its p110-inhibitory activity (Jaiswal et al 2016) as well as loss of function of the lipid phosphatases PTEN (Li et al 1997; Steck, 1997) and INPP4B (Donald et al., 2004) can all promote PI3K activity and cellular transformation. Mutations in genes acting both upstream of PI3Ks, such as RTKs (for example EGFR and HER2) and the PI3K activator K-RAS as well as the downstream PI3K effectors AKT1-3, PDK1, TSC1/2, and mTOR, are variably present in human tumors and should also result in aberrant activation of the PI3K pathway. We recently investigated the role of several natural products in attenuating PI3K pathway in several types of cancer (Badr et al.,2010; Badr et al.,2018).

Activation of PI3K pathway in Breast Cancer

Activation of PI3K pathway is aberrantly activated in breast cancer, with the two most observed mechanisms of activation being RTK signaling via amplification/overexpression of Her2 and somatic mutations in specific components of the PI3K pathway (Baselga, 2011; Yuan, 2008). Breast cancer subtypes includes ER, progesterone receptor (PR), and Her2 can have varying and unique mechanisms of PI3K pathway alterations, resulting in potentially different clinical manifestations. Thus, it has become necessary to identify and classify PI3K pathway aberrations according to breast cancer subtype. RTKs: in breast cancer, overexpression, amplification, or mutation of an RTK can directly lead to constitutive activation of the PI3K pathway. Examples in human cancers include overexpression of fibroblast growth factor (FGF) and its corresponding receptor (FGFR), gain of function mutations of EGFR, and amplification of HER2 (Ghayad and Cohen, 2010).

Somatic alterations: Loss of Pten function has been documented in a variety of carcinomas through sporadic loss of function mutations, hetero- or homozygous deletion, and/or epigenetic silencing (Sanal and Sellers, 2004). In addition, a congenital predisposition to breast cancer, known as Cowden's syndrome, is caused by germline mutations in PTEN (Liaw et al., 1997). This loss of Pten protein in tumors results in accumulation of PI (Vanhaesebroeck and Waterfield, 1999; Thorpe, 2015; Philp et al., 2001) P3 and, consequently, increased activation of Akt. Indeed, recent studies have suggested that loss of Pten leads to activation of the PI3K pathway that is dependent upon p110β (Wee et al., 2008; Jia et al., 2008). Along these lines, some studies have reported that loss of Pten reduces the efficacy of RTK inhibitors as single agents (Ghayad and Cohen, 2010) suggesting loss of Pten activates the PI3K pathway distal to RTK activation. Moreover, PIK3CA has a reported overall mutation rate commonly occur in ER (+) or HER2 amplified breast tumors, and this has been speculated to

be a major determinant of resistance to endocrine and Her2 targeted therapies (Miller et al., 2011). The AKT family of genes encodes three isoforms: Akt1, Akt2, and Akt3. For Akt1, within the lipid-binding PH domain, a somatic mutation recently identified in 8% of breast cancers (Carpten et al., 2007), resulting in a glutamic acid to lysine substitution at amino acid 17 (E17K) likely leads to transformation due to constitutive localization to the membrane, even in the absence of PI (Vanhaesebroeck and Waterfield, 1999; Thorpe, 2015; Philp et al., 2001) P3. Conversely, amplification of the Akt2 gene has been detected in 4% of breast cancers while overexpression was found in 10-20%, which corresponds to increased kinase activity in these tumors (Sun et al., 2001).

PI3K Pathway Activation in Prostate Cancer
Aberrations in PI3K/AKT/mTOR signaling have been identified in approximately 40% of early prostate cancer cases and 70–100% in advanced disease (Carver et al., 2011; Taylor et al., 2010). In particular, loss of PTEN leading to constitutive activation of the PI3K pathway has been documented in 30% of primary and 60% of castrate-resistant prostate cancers (Vivanco and Sawyers, 2002). Activation of the PI3K pathway is associated with resistance to androgen deprivation therapy, disease progression and poor outcomes in prostate cancer (Bitting and Armstrong, 2013). Over-activation via PTEN loss has been shown to initiate prostate cancer development. Varying rates of prostatic hyperplasia and cancer are seen in mouse models with heterozygous loss of PTEN (Di Cristofano, et al., 1998) and combined deletion of a second tumor suppressor gene can induce prostate cancer with complete penetrance in some models (Di Cristofano, et al., 1998). Conditional PTEN knockout mice though can mimic the course of human prostate cancer with progression from hyperplasia to invasive cancer to metastatic disease (Wang et al., 2008). Moreover, pre-clinical data demonstrate that some PTEN-deficient neoplasms, including prostate cancer,

particularly activate the PI3K pathway through the p110β isoform of the PI3K catalytic subunit (Jia et al., 2008). Ablation of p110β but not p110α inhibits downstream AKT signaling resulting in reduced tumorigenesis in these models. The association of PI3K pathway activation with castrate-refractory disease suggests that a critical component of the poor prognostic value of PI3K aberrations may be its interaction with androgen signaling. Additionally, responses to AR inhibitors in prostate cancers with PTEN loss may depend on the level of PI3K pathway activation.

Carver et al. first demonstrated in a series of studies on PTEN deficient murine and human cell lines that pharmacological PI3K inhibition increased AR protein thereby activating AR-related gene expression through a HER3 dependent mechanism (HER2 and Her3 promote AR activity and stability); similar effects were seen with AKT inhibition (Carver et al., 2011). Thereafter, they also demonstrated the inverse relationship with AR inhibition being associated with up regulated AKT signaling as a result of increased phosphorylation of AKT target genes such as GSK-alpha and PRAS40. The mechanism was determined to be through AR inhibition causing down-regulation of the androgen dependent immunophilin FKBP5 that in turn is a chaperone for the AKT phosphatase PHLPP (Pei et al.,2009).

PI3K/AKT/mTOR Pathway in Multiple Myeloma (MM)
In MM, activating mutations in PI3K and AKT have not been identified (Ismail et al., 2010). Similarly, deficiencies in phosphatase and tensin homolog (PTEN) also occur in a very small proportion of MM patients (Chang et al., 2006). In spite of all this, the pathway is activated and is of importance for MM cell survival and growth. MM is predominantly a cancer localized to the bone marrow where the cancerous plasma cells remain in close direct and indirect communication with other non-tumor cells in the bone marrow

microenvironment like stromal and endothelial cells. These interactions lead to increased secretion of interleukin 6 (IL6) by the stromal cells and vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF) by MM cells. These cytokines activate their respective receptors expressed on MM cells to up regulate signaling events such as the PI3K/AKT/mTOR, mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (MEK/ERK) and janus kinase/signal transducer and activator of transcription (Jak/Stat) pathways and promote tumor growth (Ramakrishnan and D'Souza, 2016). The complexity of the PI3K/AKT/mTOR pathway is amplified by the presence of numerous feedback loops that exist within this pathway and the crosstalk with numerous other pathways.

In addition to the above-mentioned feedback loops, MM cells modulate the PI3K/AKT/mTOR pathway through other unique mechanisms. Peterson et al. identified an mTOR interacting protein DEP domain containing TOR interacting protein (DEPTOR) that was able to inhibit both mTORC1 and mTORC2 (Pei et al., 2009). As expected, DEPTOR was expressed at low levels in various tumors. Surprisingly, DEPTOR was highly expressed in MM cells. The authors found that overexpression of DEPTOR inhibited mTORC1, which then relieved the feedback inhibition of ribosomal protein S6 kinase-insulin receptor substrate 1 (pS6K-IRS1) causing activation of AKT and survival of MM cells. Interestingly, DEPTOR overexpression was confined to myeloma cells with translocations involving cyclin D1, D3, c-MAF, or MAFB (Peterson et al., 2009). Fernandez-Saiz et al. investigated how mTORC1 and 2 interacting proteins telomere maintenance 2 (Tel2) and Tel2 interacting protein 1 (Tti1) modulate mTOR levels during starvation (Fernandez-Saiz V, Targosz BS, Lemeer S, et al., 2013). In that study, they observed that during energy crisis, Casein Kinase 2 (CK2) phosphorylates Tel2 and Tti1 specifically within mTORC1 marking them for degradation by the

F-box containing protein Fbxo9. The final result due to these events was mTORC1 inactivation and increase in mTORC2 mediated AKT phosphorylation and survival. The authors observed high levels of Fbxo9 expression in a fraction of MM patients and cell lines. Knockdown of Fbxo9 decreased the mTORC2/AKT signaling and survival of these cells. Interestingly, Fbxo9 overexpression was found to occur in hyperdiploid MM and not in the other subgroups (Fernandez-Saiz et al.,2013). Thus, DEPTOR and Fbxo9 overexpression appear to be mutually exclusive events and represent two unique mechanisms through which the PI3K/AKT/mTOR pathway gets activated in a large proportion of MM patients. In addition, other mechanisms such as high basal levels of endoplasmic reticulum (ER) stress and glucose regulated protein 78 (GRP78) cell surface localization could also contribute to constitutively activated AKT signaling in MM cells (Ramakrishnan et al., 2016). Finally, the epigenetic changes could also contribute to activation of this pathway in MM cells (Du et al., 2015).

CONCLUSIONS

In conclusion, the PI3K signaling pathway has attracted a great deal of interest due to its involvement in a large fraction of human tumors, and several inhibitors targeting oncogenic kinases throughout the pathway are currently being tested in clinical trials. In this context, it is important to identify the patients most likely to benefit from PI3K inhibitors and combinations of PI3K inhibitors with chemotherapy and other targeted therapy in the treatment of the many different types of cancers that are driven by multiple oncogenic pathways.

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