



REVIEW ARTICLE

Decrypting a path based approach for identifying the interplay between PI3K and GSK3 signaling cascade from the perspective of cancer

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Abstract Cancer is one of those leading diseases worldwide, which takes millions of lives every year. Researchers are continuously looking for specific approaches to eradicate the deadly disease, ensuring minimal adverse effects along with more therapeutic significance. Targeting of different aberrantly regulated signaling pathways, involved in cancer, is surely one of the revolutionary chemotherapeutic approach. In this instance, GSK3 and PI3K signaling cascades are considered as important role player for both the oncogenic activation and inactivation which further leads to cancer proliferation and metastasis. In this review, we have discussed the potential role of GSK3 and PI3K signaling in cancer, and we further established the crosstalk between PI3K and GSK3 signaling, through showcasing their cross activation, cross inhibition and convergence pathways in association with cancer. We also exhibited the effect of GSK3 on the efficacy of PI3K inhibitors to overcome the drug resistance and preventing the cell proliferation, metastasis in a combinatorial way with GSK3 inhibitors for a better treatment strategy in clinical settings.

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Introduction

Cancer is the leading cause of morbidity and mortality across the world. In 2020, approximately 19.3 million new cancer cases were diagnosed worldwide, with over 10.0 million cancer deaths.¹ In India the projected cancer incidence among males is 679,421 (94.1 per 100,000) and among females 712,758 (103.6 per 100,000).² The actual cause of cancer cannot be summarized as it is induced by a variety of pharmacological variables, including genetic changes, modifications of signaling pathways and the production of numerous proteins essential for the regulation of normal cellular activities.

The recent treatment strategies for the cancer include chemotherapy, radiotherapy and surgery which come with a number of side effects such as cancer relapse, drug resistance and indiscriminate destruction of non-cancerous cells. So the advance strategies mainly focused on the targeted therapy towards specific proteins to eliminate nonspecific lethality and cytotoxicity towards the healthy cells. The targeted cancer chemotherapy has gained popularity since the approval of imatinib, a tyrosine kinase inhibitor, used for the treatment of chronic myelogenous leukemia (CML).³ Following this innovation every attempt to introduce a novel molecule for targeted therapy resulted in unavoidable adverse effect, particularly drug resistance.⁴ This event has sparked an interest in exploring new sites for targeted chemotherapy that do not acquire drug resistance, which demands a detailed understanding of the targets as well as the signaling pathways that may adapt to build resistance to that specific protein of interest.

The incidence of cancer progression is associated with the oncogenic mutations which causes the over expression of mutated genes along with the deregulation of several mutated proteins. Among these large number of cancer modulated signaling pathways and proteins, phosphatidylinositol-3 kinase (PI3K), a functional protein, over expressed in most of the human cancers, which is directly involved in the regulation of cellular growth and cell survival.^{5,6} Numerous studies have been conducted on preclinical models of human cancers using the particular PI3K inhibitor, with good anticancer efficacy, but only a few of them were allowed for chemotherapeutic study as clinical trials, with disappointing outcomes due to drug resistance.^{7,8} This network seems to be regulated through a number of signaling pathways among which GSK3 signaling is important in the cancer chemotherapeutic study and also act as a potential target in the treatment of cancer.

Glycogen synthase kinase-3 (GSK3) is ubiquitously expressed serine/threonine kinase, and a multifunctional monomeric protein. This is responsible for the regulation of various cellular functions such as differentiation, survival, glycogen metabolism, protein synthesis, immune responses and cell death. This is an intermediate protein component of several signaling pathways including Wnt/ β -catenin, Hedgehog, nuclear factor κ B (NF- κ B), and transforming growth factor- β (TGF- β) signaling pathways and also plays important role as a key mediator of Insulin/Insulin receptor (IR), Insulin-like growth factor (IGF)/IGF receptor (IGFR).⁹ As a multifunctional protein, the role of GSK3 in cancer has

been assessed through a number of preclinical chemotherapeutic studies, which suggest the dual role of GSK3 in cancer. The GSK3 β , a pivotal isoform of GSK3, is a key regulator of PI3K and Wnt signaling pathway which are directly involved in the cancer development. On the other hand, activation of GSK3 β caused ubiquitin mediated proteosomal degradation of phosphorylated β -catenin¹⁰ and thus showing anticancer activity through the inhibition of Wnt- β -catenin signaling pathway.

Further research instigated the modulation of mTOR signaling via GSK3 signaling pathway in the context of targeted cancer chemotherapy. The influence of GSK3 activation and inactivation on drug resistance to mTOR inhibitors was revealed, which included a thorough molecular interaction between these two signaling pathways.¹¹ In the regulation of mTOR signaling, PI3K plays a pivotal role which is also associated with the cancer cell survival and proliferation but there is lack of crosstalk between PI3K signaling and other signaling pathway in relation to the cancer chemotherapeutics.

On the basis of recent findings, the proposed study emphasized the impact of GSK3 inducer and inhibitor on the efficacy of PI3K inhibitor through cross activation and cross inhibition of the protein component related to both of the signaling pathways for effective targeted therapy of cancer.

PI3K

The PI3K is an important component of the PI3K/Akt/mTOR pathway which is a crucial intracellular signaling, mainly responsible for the cellular growth, cell differentiation, cell survival, cellular metabolism and reorganization of cytoskeleton¹² (Fig. 1). This signaling pathway is stimulated by a number of oncogenic proteins and growth factor receptors which include insulin receptor tyrosine kinase (InsR), the related insulin-like growth factor 1 receptor (IGF-1R), epidermal growth factor (EGF), platelet-derived growth factor receptors (PDGF-R).¹³

Structure and activation

PI3K is an intracellular phosphatidylinositol kinase. The PI3K family of enzymes can be divided into three classes which are based on their structure and preference of substrates. The class I PI3Ks are the heterodimers which consists of regulatory and catalytic subunits that can be further classified into class IA and class IB according to the type of subunits.¹⁴ Class II PI3Ks are basically monomers which are composed of one of three different 110-kDa catalytic isoforms such as C2 α , C2 β and C2 γ . Class III PI3Ks are composed of heterodimeric structure which consists of a regulatory subunit Vps15/p150 and a catalytic subunit Vps34.¹⁵ The main substrate of PI3K is phosphatidylinositol (PtdIns) lipid matrix such as phosphatidylinositol-4-phosphate (PIP) and phosphatidylinositol-4,5-diphosphate (PIP2).¹⁶ After the activation of PI3K through various extracellular stimuli, such as growth factors, cytokines, and hormones,¹⁷ it causes the phosphorylation of PIP2 to PIP3

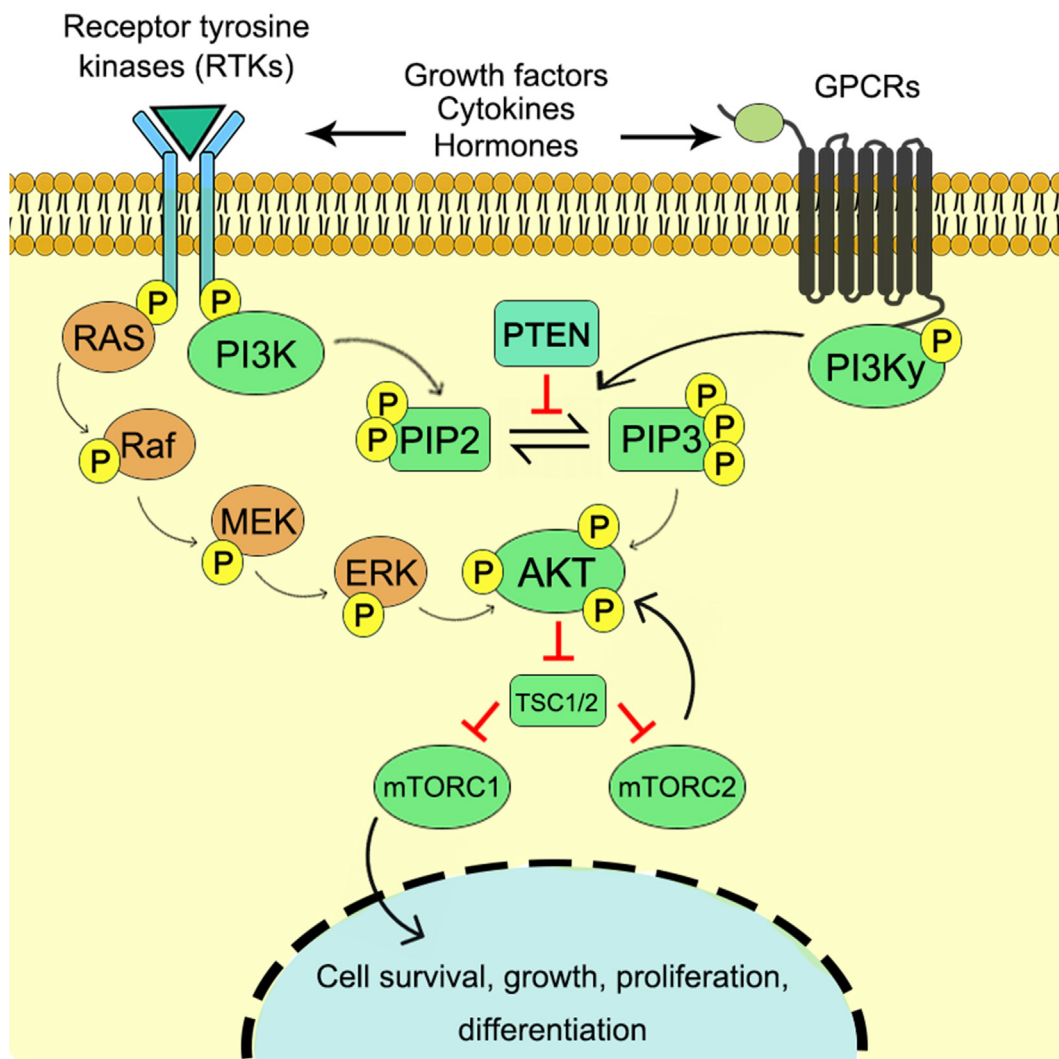


Figure 1 PI3K/Akt/mTOR signaling cascade towards cell growth, proliferation and differentiation.

and recruits several lipid binding domains to the cell membrane for downstream cascade. This in turn activates other signaling proteins such as kinases AKT and PDK1, which binds to the lipid products of PI3K and thus activates cell growth and cell survival pathways.¹⁸ Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is an important protein component for the regulation of PI3K signaling pathway through the dephosphorylation of PIP3 to PIP2 and inhibits the activation of downstream kinases.¹⁹

Oncogenic activation

Several oncoproteins and tumor suppressors are involved in cellular metabolism and regulate various signaling pathways in conjunction with PI3K signaling to maintain cellular equilibrium, which is hampered in several cancer microenvironments due to the activation and inactivation of these interrelated proteins.^{18,20} The alteration of PI3K signaling pathway is one of the primary reasons for cancer initiation due to oncogenic mutation and inactivation of tumor suppressor proteins through genetic remodulations.²¹

Mutation of PI3K

The mutation in the PI3K, more specifically at the *PIK3CA* gene encoding site that encodes for the catalytic subunit of Class IA PI3K, p110 α , is a major reason for the alteration of PI3K signaling and cancer propagation.^{22,23} Generally most of the mutation in PI3K appears around two hotspots: E545K (exon 9) in the helical phosphatidylinositol kinase homology domain, which suppresses the p85 mediated inhibition of p110 α ; H1047 (exon 20) near the end of the catalytic domain enhancing the interaction between p110 α and lipid membranes.²⁴ Along with that, mutation on the C2 domain is also significant for the *PIK3CA* mutation.²⁵ On the other hand mutations on the other catalytic subunits such as p110 β , p110 γ and p110 δ are rare, which are also responsible for the induction of oncogenic phenotype.²⁶

PTEN inactivation

PTEN, a tumor suppressor protein, regulates the cell survival and cellular metabolism. Inactivation of this negative

regulatory protein has been associated with both heritable and sporadic malignancies such as breast cancer, and prostate cancer.^{27,28} Any alteration in the expression of PTEN showed a major impact in the normal cellular function. According to the research conducted by Papa and his co researchers, a mutation on PTEN causes significant inhibition of PTEN lipid-phosphatase activity which leads to the enhanced activity of PI3K signaling and ultimately tumorigenesis. The carcinogenic outcome corresponds to the over activity of Akt due to the inhibition of PTEN lipid-phosphatase activity.²⁹

Regulation of non-coding RNA (ncRNAs) and other factors

It has been observed that the PI3K signaling pathway is activated through the lncRNA CRNDE and thus promotes cell proliferation with high expression of the ncRNA in various cancer patients with non-small cell lung cancer, colorectal cancer, gastric cancer, cervical cancer, heptaocellular carcinoma and gallbladder cancer.^{30,31} The ncRNA is also shown to have inhibitory effect on the PI3K signaling pathway. In the tumor microenvironment the lncRNA GAS5 expression is lower and the over-expression of it inhibits cancer cell proliferation and migration.³²

Shaping of tumor microenvironment (TME)

The tumor microenvironment is composed of heterogeneous cell population such as endothelial cells, pericytes, cancer associated fibroblasts (CAFs), stromal cells and host immune cells including tumor-associated myeloid cells.³³ These large numbers of cellular components interact with each other via a complicated network that consists of cytokines, mitogens and growth factors which facilitates tumor growth and proliferation. In this regard, PI3K signaling plays a significant role in the oncogenic activation through proliferation, differentiation and migration of these cells thus facilitating the modulation of TME.³⁴

PI3K in angiogenic activation

In the process of tumor growth and metastasis, angiogenesis plays an important role through the sufficient blood supply at the TME. The process of angiogenesis in the TME is regulated by a number of signaling pathways in which the cancer cells can elicit the vascular growth through the modulation of the angiogenic equilibrium maintained by several signaling molecules. In the activation of angiogenic response, PI3K signaling plays a multifaceted role in both malignant and nonmalignant cells.³⁵ The activation of the angiogenic response in the TME is stimulated by hypoxia and nutrient deprivation due to rapid tumor growth which causes the secretion of proangiogenic growth factors and cytokines. As per the previous studies, the activation of PI3K signaling is important for the hypoxia-inducible factor (HIF)-1 α -mediated secretion of VEGF.³⁶ Inactivation of PI3K signaling through the expression of PTEN or a dominant-negative mutant Akt can downregulate the HIF-1 and VEGF expression in TME.³⁷ According to a murine study, it

has been showed that activation of PI3K p110 γ promotes the myeloid cell trafficking to tumors, which facilitates tumor growth, invasion, angiogenesis and metastasis.³⁸

PI3K in remodeling of extracellular matrix (ECM)

In the TME, the ECM is mainly secreted by cancer associated fibroblasts (CAFs) which provides structural scaffolding with biochemical and biomechanical cues surrounding cells thus leads to the cell differentiation, proliferation and migration. CAF is an essential component of ECM which is responsible for the cancer progression and metastasis.³⁹ Various PI3K associated pathways including TGF- β , HGF, EGF and PDGF signaling pathways are closely related with the stromal cell differentiation into CAFs and CAF activity.^{40,41} Another essential factor for the ECM remodeling is matrix metalloproteinase (MMP), which are also associated with the activation of PI3K signaling.⁴²

PI3K in autophagy regulation

The process of autophagy can be defined as a recycling process that is associated with the breakdown of the proteins and cellular organelles to their macromolecular precursor. TE cells undergoes autophagy in response to the cellular stress and which may lead to both the cellular death or cell survival depending upon the circumstance.⁴³ The PI3K pathway has been identified as the major regulatory pathway for autophagy.⁴⁴ The mTORC2 protein is directly involved in the regulation of autophagic proteins which is a direct downstream regulatory component of PI3K signaling axis.⁴⁵ Besides that, the PI3K pathway also plays an important role in the integration of the cellular signals in association with several other pathways including Ras/Raf/MEK/ERK and MAPK/JNK pathways to regulate the autophagic responses.⁴⁶ The initiation of autophagy occurs in two ways by the action of Unc-51-like kinase (ULK) complex. First, in the vicinity of membraned organelles the ULK complex gets accumulated. Second, the ULK complex binds with the ubiquitinated cellular components. Both the pathways ultimately lead to the accumulation and activation of ULK complex and triggers autophagy.⁴⁷ The mTOR protein involved in the inhibition of this complex and I3K pathway regulates the fate of autophagic response extensively.⁴⁸ The PI3K pathway is also involved in the ROS mediated regulation of autophagy which maintains ROS homeostasis subjected to cellular growth and proliferation.⁴⁹

Regulation of cancer stemness

In the TME, among various types of cells, cancer stem cells (CSCs) are very unique subpopulation, which are self-renewable and responsible for tumor recurrence, metastasis, and progression.⁵⁰ Dedifferentiation and regulation of stemness is mostly linked to the expression of PIK3CA cancer hotspot variant, H1047R, in various cancer model systems such as breast,⁵¹ lung,⁵² colorectal cancers.⁵³ The activation of EMT, epithelial-to-mesenchymal transition, is

also linked with the oncogenic activation of PI3K α .⁵⁴ The development of autocrine signaling loops, particularly the TGF β pathway mediated pro-tumorigenic action, is thought to constitute a link between cancer stemness and EMT.^{55,56} In cancer-relevant cell models, there is substantial evidence for a connection between TGF β and PI3K signaling in the stemness regulation.⁵⁷

Thus, the role of PI3K signaling in the cancer microenvironment is obvious from the aforementioned findings, suggesting this protein as an important target for targeted chemotherapeutic research. Though PI3K has some anti-cancer immune responsive function, the impact on suppressive immune cell subsets outweighs the benefits of a stronger immune response against cancer and certain infectious pathogens.⁵⁸ Considering this, a substantial number of PI3K inhibitors have already received approval for clinical trials.⁵⁹

The PI3K pathway and drug resistance

The PI3K signaling pathway and its downstream signaling components have been identified as important targets in the development of chemoresistance against cancer therapy in a number of malignancies.^{60,61} Abnormal activation of this signaling pathway causes inhibition of chemotherapy induced apoptosis via distinct mechanisms such as activation of anti-apoptotic genes and inactivation of pro-apoptotic genes thus develops drug resistance.⁶² Activation of Akt triggers PAK1 and phosphorylates Bad protein which released from mitochondrial membrane to cytoplasm and on the other hand prevents the translocation of Bax from cytoplasm to mitochondria. Therefore, abnormal activation of Akt stimulates the cell survival through the stimulation of Bcl2 and inhibition of Bax.^{63,64} The alteration of PI3K signaling pathway also stimulates the expression of X-linked inhibitor of apoptosis (XIAP), a downstream effector of Akt, which inhibit apoptosis through the regulation of cytosolic p53 level.⁶⁵ The overactivation of NF- κ B system by the alteration of PI3K signaling network also leads to tumor growth and apoptosis inhibition. The NF- κ B pathway stimulates the process of cell cycle and thus causes development of multidrug resistance in cancer cells.^{66,67} The inactivation of FOX factors due to abnormal activation of PI3K signaling cascade also leads to drug resistance.⁶⁸ The overactivation of Akt1 stimulates HER2/PI3K signaling which is responsible for chemoresistance in breast cancer.⁶⁹ The deregulation of micro-RNA also exerts a significant effect in the development of drug resistance.⁷⁰

GSK3

The first ever identified GSK3 was introduced in 1980s for their function in insulin signaling, since then GSK3 has been studied and found to be mediated by different molecular pathways such as Notch signaling, Wnt signaling, phosphoinositide 3-kinase signaling pathway.^{71,72} The schematic diagram representing the detailed GSK3 signaling cascade has been depicted in Fig. 2. GSK3 has significant involvement in different cellular pathways including PI3K/Akt/mTORC1, Wnt and Hedgehog signaling. Thus any alteration of GSK3

leads to different disease conditions such as diabetes, cardiovascular diseases and cancer.^{73,74}

Structure and regulation of GSK3

GSK3 is a member of CMGC family, a reserved serine/threonine kinase. In eukaryotes GSK3 α (51 Da) and GSK3 β (47 kDa) are encoded by *GSK-3A* and *GSK-3B* genes.⁷⁵ GSK3 is made up of two domains: a strand with seven anti parallel β -strands interrupted by a short helix of highly conserved regions and α -helix. A hinge region and a glycine-rich loop surround the catalytic site at the interface of the two domains.⁷⁶ These two isoforms share 98% similarity in their kinase domain and 36% in their C-terminal. Additionally, GSK3 α is comprised of a glycine contained extensions at their amino terminal which lacks in GSK3 β . These isoforms can be divided based on their cellular functions, patterns and substrate of interest.⁹

GSK3 activation and inactivation

The activation and inactivation of GSK3 is controlled by the post translational, site specific phosphorylation of serine and tyrosine residues. In normal resting cells, GSK3 α and GSK3 β remain active. They enhance the catalytic action at the position of Y279 and Y216, which has a constitutive function in resting cells.⁷⁷ Additionally, it has been shown that Y216 phosphorylation is also induced by apoptotic stimulus.⁷⁸ Sometimes, proline rich tyrosine kinase 2 and Fyn tyrosine kinase helps in the phosphorylation and causing the activation of GSK3.^{79,80}

On the other hand, activation of insulin mediated phosphatidylinositol 3-kinase (PI3K), PDGF and EGF helps in the inactivation of GSK3. Besides that, upregulatory kinases phosphorylate GSK3 β at S9 residue and inactivate GSK3 including protein kinase A, protein kinase B/Akt, mitogen activated kinase.^{81,82} Followed by the activation, GSK3 phosphorylate different downstream substrates. GSK3 α and GSK3 β have greater affinity towards the substrates which are already phosphorylated by different protein kinases (priming kinases). Additionally, the substrate which contains priming phosphate gets phosphorylated by GSK3 easily than that which lacks priming phosphate.⁷²

GSK3 in cancer

GSK3 signaling has been found to be associated with several intracellular pathways which attribute to different modes of oncogenic regulation. GSK3 involves in the process of cell survival through the regulation of NF- κ B pathway and repress Wnt/ β -Catenin, showing tumor suppressor activity. GSK3 is also associated with Wnt and Hedgehog pathway, promoting variety of cancers such as melanoma, hepatocellular carcinoma, prostate, colorectal cancer.^{83,84}

Induction and suppression of malignancy

GSK3 over expression has been found to be associated with tumor promoting activity in several cancer models. As per the earlier studies, over expression of GSK3 promotes cell

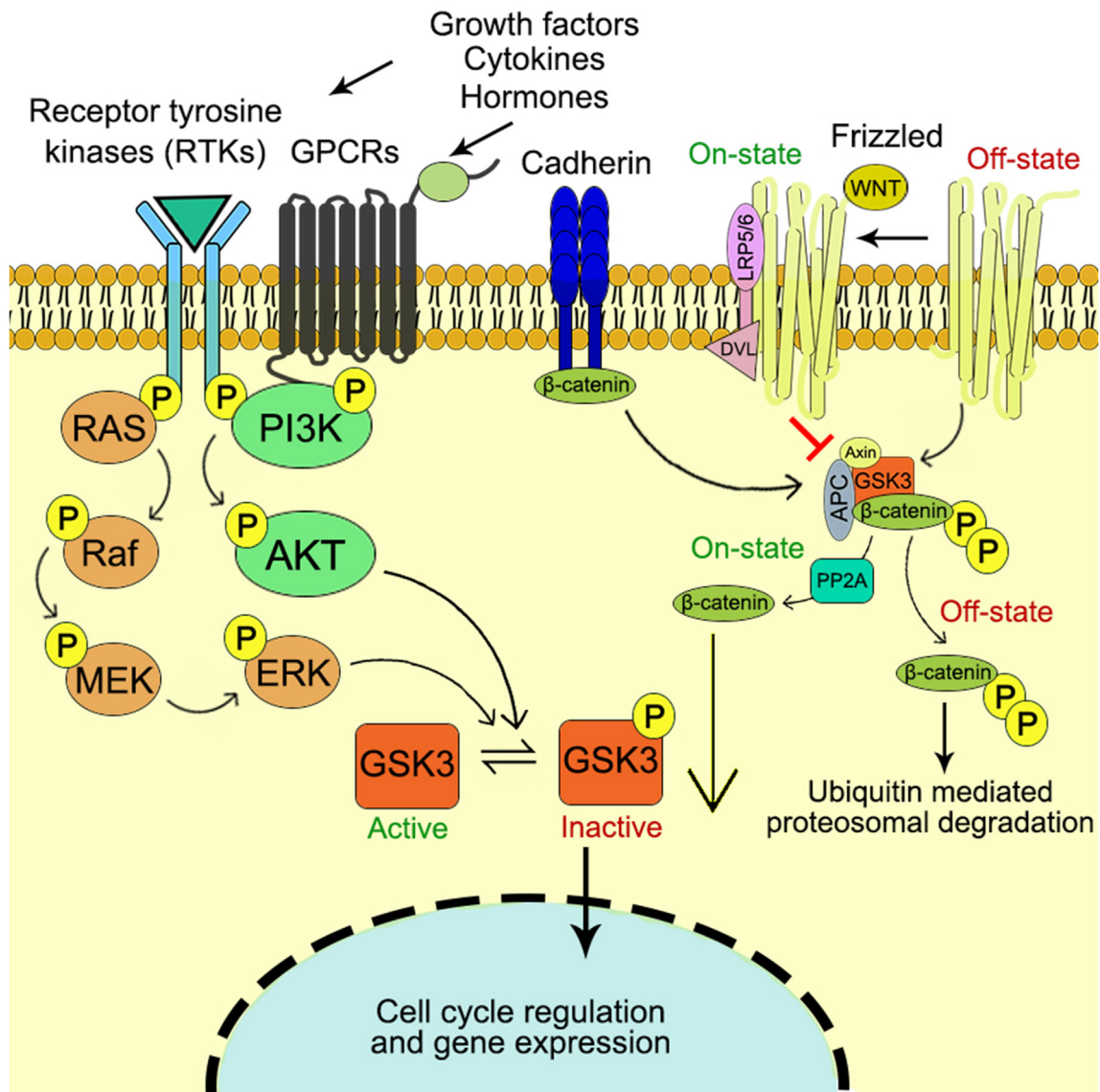


Figure 2 GSK3 signaling cascade towards cell cycle regulation and gene expression profiling.

proliferation, survival, invasion, and angiogenesis. Activation of GSK3 β induces kinase activity and tumor dedifferentiation resulting in brain cancer, along with that; it also decreases the patient survival rate in glioblastoma multiforme.⁸⁵ Low level of pSer9 GSK3 β expression along with the active GSK3 induces cellular growth in pancreatic, renal and colorectal cancer. Inactivation of GSK3 β triggers apoptosis and control proliferation in these cancers.⁸⁶ It has been also found that, GSK3 up regulation is also associated with a worse survival rate in endometrial cancer and it is positively correlated with prognosis, dedifferentiation, and myometrial infiltrate level.⁸⁷ In blood cancer, splicing of GSK3 β mRNA up regulate the β -Catenin level and activate stem cells.⁸⁸ Hence, the inhibition of GSK3 possesses a novel approaches in the cancer chemotherapy.

In contrast, tumor suppressive function of GSK3 β is also instigated through the inactivation of the related genes which causes cancer. In dermal malignancy, the over expression of pSer9 GSK3 β and low level of pTyr216 GSK3 β

was observed.⁸⁹ The mutation or inhibition of GSK3 β is also correlated with the induction of breast carcinoma.⁹⁰ The sequential expression of p521 GSK3 α and pS9 GSK3 β also contribute in the oral cancer progression.⁹¹

Cell cycle regulation

Cyclins, cyclin dependent kinase inhibitors (CDK) and check point inhibitors are important protein which has a crucial role in cell cycle regulation. GSK3 is associated with the phosphorylation of these proteins and shown to have crucial effect on the cell cycle.⁹¹ The phosphorylation of cyclin D1 at T286 and cyclin E at T380 is regulated by GSK3, which is essential for nuclear export and break down.^{92,93} Additionally, the suppression of cyclin D1/CDK4/6 complex mediated phosphorylation of Rb tumour suppressor protein leads to the inhibition of tumour GSK3 β activity.⁹³ GSK3 influences the RNA synthesis of cell cycle checkpoint

blockers such as GADD45 and GADD153 through the expression of the tumour suppressor TP53, which increases in response to DNA damage.⁹⁴

Role in apoptosis

Several proteins are involved in the cellular apoptosis which act as a potential target for GSK3 β . Among them the anti-apoptotic protein such as, few Bcl-2 sub types has been found to have great affinity towards GSK3 β .⁷⁸ Two major transcription factor, CREB factor and c-Myb get phosphorylated by the GSK3 β , which alter the Bcl-2 expression.⁹⁵ GSK3 β mediated inhibition of ubiquitin-dependent destruction of c-Myb and transcriptional activation enhancement of the Bcl-2 promotes cellular proliferation and survival of the blood cancer cell.⁹⁶ Besides that, GSK3 β also promotes the phosphorylation of Bax that leads to mitochondrial localization and trigger the proapoptotic activity in the neuronal cells.⁹⁷ From these above findings, the multi-disciplinary role of GSK3 β can be found in cellular apoptosis in different cancer model systems.

Role in autophagy

Autophagy is a complex cellular phenomenon which involved diverse molecular machinery including mTOR, a key component in the autophagy regulation.⁹⁸ mTOR senses the intracellular amino acids, ATP, hormones and eventually inhibits the process of autophagy. GSK3 also possessed a vital role in the autophagy regulation through the activation of mTORC1 and thus inhibits autophagy.⁹⁹ Additionally, the increased expression of GSK3 subunits associated with the activation of mTORC1 through the phosphorylation of mTOR associated scaffold protein Raptor at Ser859 and leads to the inhibition of autophagic responses. The inactivation of GSK3 causes inhibition of mTOR and raptor via reduced phosphorylation of both p70S6K1, ULK-1 and triggers autophagic responses.¹⁰⁰ The overexpression of GSK3 also causes an increase in the number of autophagosome through the inhibition of autophagic flux via inhibition of lysosomal acidification.¹⁰¹ Moreover, GSK3 regulates the transcription factor EB (TFEB), a key regulator of autophagy and lysosomal biogenesis.¹⁰² In prostate cancer inhibition of GSK3 β induces autophagy through the increase in AMP/ATP ratio, which triggers the activation of AMPK.¹⁰³ Along with that, GSK3 inhibits mTOR through the phosphorylation of TSC2 in an AMPK-priming phosphorylation-dependent manner.¹⁰⁴

Role in tumour invasion

The epithelial–mesenchymal transition (EMT) can be distinguished by the loss of function of E-cadherin, which is one of the initial steps in tumour invasion. GSK3 suppresses EMT by blocking Snail, which works as an E-cadherin transcriptional repressor. GSK3 mediated phosphorylation of Snail leads to the nuclear transportation, proteosomal degradation followed by elevated E-cadherin and decreased matrix metalloproteinase (MMP) expression.¹⁰⁵ Further studies showed that inhibition of both GSK3

isoforms triggered Snail expression, whereas inhibition of either isoform alone did not exhibit any effect indicating that activity of the both isoforms is necessary for the maintenance of epithelial morphology.¹⁰⁶ Besides that, GSK3 has also been demonstrated to suppress Snail transcription through the suppression of NF- κ B and β -catenin signalling molecules. Thus, the role of GSK3 in the regulation of EMT through various signalling pathways has been identified.⁹

miRNAs and GSK3

OncomiRs

miR-26a is a direct target of GSK3, which increase the metastasis in lung cancer through the inactivation of GSK3 β , which leads to the β -catenin nuclear translocation and enhance the regulation of C-myc and cyclin D1.¹⁰⁷ On the other hand, the over activation of GSK3 β can alternate the oncogenic activity of miR-26a. In mammary carcinoma, GSK3 β get targeted by the over activated miR-1229 and increase proliferation and trigger wnt/ β -catenin pathway.¹⁰⁸ There are various oncomiRs which play significant roles in different ways.^{109,110}

Tumour suppressor miRs

According to dual-luciferase and xenograft reports from the bioinformatics study, GSK3 β might be a potential target of miR-129 in the endometrial carcinoma. The GSK3 β inhibitor AZD1080 protect the endometrial cells from the uncontrolled growth, invasion and regulate the apoptosis via GSK3 β targeted genes.⁸⁷ In oral cancer, both miR-99b-3p expression and GSK3 β silencing are crucial for the prevention of oral carcinoma through the suppression of GSK3 β .¹¹¹

Stemness

Stem-cell-like phenotype promotion is one of the functions of overexpressed miR-744 in case of pancreatic cancer cells. These miR-44 overexpression targets GSK-3 β to enhance Wnt/ β -catenin signaling.¹¹² In case of CD133 liver cancer stem cells, miR-1246 can promote the Wnt/ β -catenin pathway and reduce GSK3 β activity which is important for the enzyme mediated self-regulation and uncontrolled growth of cancer cells. These incidences provide new opportunities for the development of novel therapeutic approaches.¹¹³

Role in drug resistance

GSK3 singling pathway has been demonstrated to be involved in the development of chemoresistance by the action of GSK3 β particularly. In glioma, the nuclear GSK3 β is associated with induction of DNA double strand break repair through the phosphorylation of 53BP1 and thus promotes chemoresistance.¹¹⁴ Temozolomide is a first line chemotherapeutic drug to treat glioblastoma. GSK3 β causes methylation of O6-methylguanine DNA methyltransferase promoter which sensitizes the cancer cells against

temozolomide.¹¹⁵ Additionally, GSK3 β phosphorylates the Akt and PTEN which further initiates chemoresistance in breast cancer.¹¹⁶ In colorectal cancer, the overexpression of NF- κ B mediated through the GSK3 β activity modulates the regulation of cancer stem cells to develop drug resistance.¹¹⁷ The function of GSK3 β is associated with the activation of ATR-interacting protein TopBP1 and activation of ATR in single strand DNA damage repair which desensitizes the pancreatic ductal to chemotherapy and promotes chemoresistance.¹¹⁸ The overexpression of GSK3 β associated with the development of acquired resistance in ovarian carcinoma.¹¹⁹ GSK3 β also promotes epithelial to mesenchymal and cancer stem cell properties by acting on the TGF β which promotes chemoresistance in the ovarian carcinoma.¹²⁰ Moreover, GSK3 β regulates the p53 expression and phosphorylation of Ser9 of GSK3 β (pGSK3 β Ser9) confer the cisplatin resistance of ovarian carcinomas.¹²¹ In renal carcinoma, GSK3 β causes the phosphorylation of 4EBP1 and activation of mTORC1 which triggers the renal carcinoma cells to acquire resistance against mTORC1 inhibitors.¹²²

PI3K inhibitor

In order to develop the PI3K inhibitors tremendous attempts has been made which slows tumor progression and modifies immunosuppressive tumor microenvironment. The chemotherapeutic activity of PI3K inhibitors have also been established in clinical settings (Table 1). These PI3K inhibitors are identified as the potential strategy to combat cancer which can be classified based on their selectivity such as pan-PI3K inhibitors, selective isoform PI3K inhibitors and dual PI3K/mTOR inhibitors.¹³⁵

Pan-PI3K inhibitors

Pan-PI3K inhibitors are ATP-competitive inhibitors, targeting all four isoforms of PI3K class I which leads to numerous side effects due to nonselective blockage of PI3K. In the cases of PTEN inactivation or aberrant upstream PI3K signaling they are often utilized as they effectively block the PI3K pathway.¹³⁶ Some of the pan-PI3K inhibitors are Buparlisib, PX-866, voxalisib, pilaralisib, pictilisib, Copanlisib, and ZSTK474.

Selective PI3K isoform inhibitors

Selective PI3K isoform inhibitors are the significant approach to develop ATP-competitive which is associated with minor side effects such as immunosuppression and glucose intolerance. Though they are responsible for the inhibition of selective enzymes, but they have very narrow therapeutic window due to the lack of selectivity on the mutant isoforms.¹³⁷ It can be divided based on the isoforms to 4 classes which includes selective PI3K α , PI3K β , PI3K γ , and PI3K δ inhibitors.

PI3K/mTOR dual inhibitors

It has also been shown that few PI3K inhibitors also cause the inhibition of mTOR protein due to their binding affinity

to the catalytic domain of mTOR which resembles high degree of sequence homology with p110 catalytic subunit of the class I PI3Ks. The primary advantages of these dual inhibitors are mainly targeting both the protein components at the same time with a single agent which reduces the unnecessary side effects and also improves the therapeutic outcome.¹³⁸ Some of the PI3K/mTOR dual inhibitors are currently under clinical trial program such as GSK2126458, XL-765, GDC-0980, PKI-587, PF-06491502, BEZ235 and BGT226, among which GSK2126458, BEZ235 and BGT226 showed excellent chemotherapeutic outcome *in vitro* and *in vivo*.¹³⁹

Toxicity and adverse reactions

Besides all the potent clinical achievements the PI3K inhibitors comes with potentially fatal adverse reactions. The most frequent adverse events that have been noticed during the treatment with PI3K inhibitors include nausea, vomiting, fatigue, cough, fever, headache and anorexia which are not at all serious adverse effects sufficient for termination of the therapy and can be managed clinically. The serious adverse effects for the patients treated with idelalisib plus rituximab were pneumonia, diarrhoea, pyrexia, sepsis, and febrile neutropenia which lead to discontinuation of the therapy. The most common side effects for the termination of the therapy are hepatotoxicity and diarrhoea/colitis.¹⁴⁰

GSK3 inhibitor

There are different types of GSK3 inhibitors such as ATP-competitive GSK3 inhibitor, non-ATP-competitive GSK3 inhibitor, substrate competitive GSK3 inhibitor, isoform-specific GSK3 inhibitor, combination with GSK3 inhibitor, and multikinase inhibitor.⁹

ATP-competitive GSK3 inhibitor

ATP-competitive GSK3 inhibitors are more available than the others. They are non-selective to two types of isoforms of GSK3.¹⁴¹ Lithium is the first approved GSK3 inhibitor, it inhibit GSK3 directly and indirectly with the help of magnesium and Akt activation, respectively.¹⁴² One of the bis-indole compounds indirubin is considered as dual inhibitor of CDKs and GSK3 β through ATP-competitive process. The indirubin derivative 6-bromoindirubine-3'-monoxime scaffolds are crucial for developing the inhibitors of GSK3 β . It also helps to downregulate androgen receptor-V7 which acts as a part of prostate cancer.¹⁴³

Non-ATP-competitive GSK3 inhibitors

Heterocyclic thiazolidinone classified small molecule, tideglusib (TDZD-8) are ATP non-competitive GSK3 inhibitors which contain thiazolidindione scaffold.¹⁴⁴ The novel substituent of benzothiazinones are reported to inhibit GSK3 β through non-ATP competitive inhibition and termed as a potent candidate for the treatment of ovarian cancer.¹⁴⁵

Table 1 Summary of trials, outcomes and adverse effects associated with PI3K inhibitors in various phases of clinical studies.

Treatment and reference	Phase and NCT	Clinical outcomes	Adverse effects
BKM120/Buparlisib			
Buparlisib in combination with Everolimus (Eve) in patients with advanced solid tumors ¹²³	I, NCT01470209	The combination was well tolerated and safe in these patients. The MTD and RP2D for Eve and Bup was 5 and 60 mg, respectively, when on continuous daily schedule. There was no evidence of drug–drug interaction with concurrent administration of Eve and Bup. Paired skin biopsies for baseline and cycle 1 patients demonstrated target engagement with modulation of mTOR/PI3K signaling pathway biomarkers. There was a marked reduction in pS6 and p4EBP1 levels in cycle 1 biopsies compared to baseline.	Diarrhea, nausea, hyperglycemia, hypokalemia, muscular pain, anorexia, fatigue and elevated ALT/AST. 7 patients had additional DLTs such as mucositis, acute kidney injury and urinary tract infection. Gr 4 and 5 adverse effects were rarely observed.
Buparlisib in combination with MEK162/Binimetinib in patients with advanced solid tumors ¹²⁴	I, NCT01363232	The combination showed promising activity in patients with ovarian cancer with RAS/BRAF mutation. The MTD for Bup and MEK162 was established at 90 mg/day and 45 mg twice daily dose, respectively. The RP2D for Bup was determined as 80 mg/day and MEK162 45 mg twice daily dose. Other dosing strategies such as pulsatile dosing should be adopted for further trials as continuous dosing led to intolerable toxicities.	Central serous retinopathy, diarrhea, stomatitis, pneumonia, vomiting, nausea, maculopapular rash, increase in ALT and elevation in blood creatine phosphokinase.
Buparlisib in combination with Temozolamide (Tem) and Radiation Therapy in newly diagnosed glioblastoma patients ¹²⁵	I, NCT01473901	Due to challenging safety profile and inability to achieve the MTD, the sponsor decided not to pursue the use of Bup in newly diagnosed glioblastoma patients.	
Buparlisib in combination with mAb targeting EGFR, Panitumumab (Pani) in patients with metastatic/advanced RAS-WT colorectal cancer ¹²⁶	Ib, NCT01591421	The combination of Bup (given 5 days a week) and Pani (6 mg/kg by IV route biweekly) was well tolerated ($n = 19$). Both drugs were administered in 3 DLs. The median PFS was 2 months. 9 patients had PD and 7 patients exhibited SD (the median duration was 5.4 months [range: 3.7–8.4 months]). The phase II study was stopped, as the predefined futility rule for response was not achieved.	mucositis, fatigue, palmar-plantar erythrodyesthesia, rash, acneiform, hypomagnesemia and increased AST/ALT.
Buparlisib in combination with tyrosine kinase inhibitor, Imatinib in patients with gastrointestinal stromal tumor for whom treatment failed prior to Imatinib and Sunitinib therapy ¹²⁷	Ib, NCT01468688	The combination failed to provide additional benefits compared to current therapies that are available for these patients ($n = 60$). Further development of this combination was terminated due to lack of objective response.	

Table 1 (continued)

Treatment and reference	Phase and NCT	Clinical outcomes	Adverse effects
Buparlisib in combination Carboplatin or Lomustine in patients with recurrent glioblastoma multiforme ¹²⁸	Ib/II, NCT01934361	The combination did not demonstrate sufficient anti-tumor efficacy compared to single-agent Lom or Carbo. The study did not proceed to phase II trial.	
Buparlisib in R/R CLL patients ¹²⁹	II, NCT02340780	Bup demonstrated significant toxicities and further testing of Bup in these patients was ceased ($n = 14$). The data also indicated that basal raptor expression in CLL patients correlated with clinical response to Bup.	
Buparlisib in TNBC patients ¹³⁰	II, NCT01790932 and NCT01629615	No confirmed objective responses were observed and Bup was not associated with a strong clinical efficacy in TNBC patients as a single agent ($n = 50$).	
Buparlisib in combination with LGX818/ Encorafenib (BRAF inhibitor) and MEK162/ Binimetinib (MEK inhibitor) in patients with advanced BRAFV600-mutant melanoma (LOGIC-2) ¹³¹	II, NCT02159066	The triple therapy was feasible depending on genetic alterations but low clinical activity was observed ($n = 6$). Further exploration is needed to identify the patterns of resistance susceptible to use this combination in these patients.	
Copanlisib/BAY 80-6946/Aliqopa			
Copanlisib plus the MEK inhibitor, Refametinib (Ref) in advanced cancer patients ¹³²	I, NCT01392521	In this dose-escalation ($n = 49$) and expansion ($n = 15$) study, the MTD was defined at Cop 0.4 mg/kg weekly and Ref 30 mg twice daily dose. There was no drug–drug interaction observed. MEK-ERK inhibition and decreased tumor FDG uptake were reported during treatment. Best response was SD in $n = 21$ patients. PRP pAKT levels demonstrated sustained reductions from baseline post-Cop treatment [median inhibition: 0.4 mg/kg, 73.8% (range-94.9 to 144.0); 0.8 mg/kg, 79.6% (range-96.0 to 408.0)]. Tumor pAKT was lowered versus baseline with Cop 0.8 mg/kg in paired biopsy samples. Dose-related transient plasma glucose elevations were seen. Cop plasma exposure significantly correlated with alterations in plasma pAKT levels and glucose metabolism markers.	Fatigue, diarrhea, nausea and acneiform rash. DLTs included oral mucositis increased ALT/AST, rash acneiform, hypertension and diarrhea.
Copanlisib in patients with solid tumors and NHL patients ¹³³	I, NCT02155582		Hyperglycemia, fatigue and hypertension.
Copanlisib in Chinese patients ¹³⁴	I, NCT03498430	Cop PK exposure profiles were in the same range as of those from previous studies of Cop in non-Chinese patients in clinical dose of 60 mg. The ORR was 50% (95% CI: 21.1, 78.9) with 6 patients achieving a best response of a PR in 1 patient and SD in 6 patient.	Hyperglycemia, transient hypertension both Gr 3. However, no Gr 4 or Gr 5 adverse events observed.

Substrate-competitive peptide GSK3 inhibitors

Short phosphorylated peptides which are developed from pre-phosphorylated substrates function as selective substrate-competitive inhibitors. A heat shock factor-1 (HSF-1) derivative, peptide L803, is also an effective inhibitor of GSK3. Further, in the modified version of L803 (L803-mts), myristic acid was introduced to N-terminus for increasing the permeability. L803-mts is found to be more specific towards GSK3 with more reducing potential of phosphorylation of the GSK3 substrates without interfering with other protein kinases.¹⁴⁶

Isoform-specific GSK3 inhibitors

Some of the compounds have the ability to inhibit both the isoforms of GSK3. In case of acute myeloid leukemia, 'scorpion shaped' GSK3 α inhibitor are used for more specific activity which reduces unnecessary side effects.¹⁴⁷ Additionally, AR-A014418GSK3 is a specific inhibitor which can decrease the telomerase activity.¹⁴⁸

Combination therapy with GSK3 inhibitors

The demand of the combination therapy is getting more hyped and GSK3 inhibitors are not excluded from the list. GSK3 β specified inhibitors in a low dose with the combination of the different therapeutic agents are claimed to possess synergistic effects.¹¹⁵ As an example, metformin, pioglitazone and lithium comprise a triple agent dose regime which is suggested as adjuvant for metabolic activity.¹⁴⁹ GSK3 inhibitors such as BIO acetoxime, TDZD-8, L803-mts combine with some autophagy modulators like chloroquine, 3-methyladine which might be potential for treatment of malignant gliomas.¹⁵⁰

Multikinase inhibitors

Multiple kinases inhibiting compounds are being developed for better chemotherapeutic outcome. Compounds which are comprised of a central pyrazole and benzimidazole scaffold causes the inhibition or modulation of GSK3, Aurora, CDK kinases.¹⁵⁰

Toxicity and adverse effects

No such documented adverse effects are found in case of the GSK3 inhibitors as most of the inhibitors are under clinical trial process (Table 2).¹⁵¹ The specificity and safety against other kinases is a big question for the researchers. As most of the GSK3 inhibitors has a tendency to compete with ATP to get bind with GSK3, there can be a chance of the off target binding. Additionally, as GSK3 is present at the center of different crossed signaling pathways, inhibition of GSK3 can interfere with other signaling pathways which lead to undesirable output. β -Catenin activation is a topic of concern which can cause transformation of the healthy to malignant phenotype through activating different genes.⁹ In order to eradicate all probable adverse

reactions an in-depth investigation of GSK3 in both normal cell and cancerous microenvironment is encouraged.

Crosstalk between PI3K and GSK3

The PI3K and GSK3 proteins are linked to each other through various complex and imprecise signaling networks. The crosstalk between PI3K and GSK3 is associated with the unwinding of the intricate intracellular signaling by means of cross-activation, cross-inhibition and pathway convergence on substrates (Fig. 3, 4).

Cross-inhibition

The PI3K signaling pathway negatively regulates the activity of GSK3. Phosphatase and tensin homolog (PTEN) is an important negative regulator of PI3K signaling pathway. Mutation of PTEN is very commonly associated with the gliomas,^{155,156} which leads to the loss of inhibitory control over the glycolysis and thus caused the tumor invasion. The loss of negative regulation on PI3K signaling caused the phosphorylation mediated deactivation of GSK3 and allows the activation of glycogen synthase (GS). There are various other kinases that can phosphorylate and inactivate GSK3 activity such as mitogen-activated protein kinase (MAPK, ERK1/2).¹⁵⁷ However, an intimate relation between MAPK/ERK cascade and PI3K signaling justifies the effect of PI3K on GSK3 through MAPK mediated pathway. Both PI3K/Akt and MAPK plays an important role in the cell proliferation and regulation of cell cycle through the phosphorylation of various downstream effector molecules.¹⁵⁸ Mutations in the RAS genes results in the activation of both PI3K/Akt and MAPK pathways which leads to phosphorylation of GSK3 and thus inactivation. Furthermore, the mutation of RAS also linked with the activation of Akt in thyroid tumors development.^{159,160} The occurrence of prostate cancer have been distinguished by observing the progression from androgen-dependent phenotype to androgen independent (AI) and lethal. The PI3K and androgen receptor (AR) signaling pathways plays important role in the development of prostate cancer through the inhibition of apoptotic events associated with androgen withdrawal therapy. The loss of PTEN and GSK3 activity is readily associated with the induction of AR activity and PI3K/Akt gain-of-function as PTEN is a negative regulator of AR and PI3K signaling. So the loss of PTEN activity leads to accumulation of the phospholipid and results in the phosphorylation of Akt at the submembranous position¹⁶¹ which further causes phospho-inhibition of GSK3 β and inactivation of apoptotic factors. Additionally, several evidences have been suggested the role of GSK3 in the regulation of AR oncogenic function in the process of prostate cancer development. The phosphorylation of the AR hinge and ligand binding regions by GSK3 β inhibited the activation of AR-genes targets and cell proliferation.^{162,163} These evidences strongly suggesting the GSK3 regulation in the prostate cancer cells and serves as a vital pharmacological target.¹⁶⁴ Along with that, in prostate cancer the enhanced autocrine production of IGF-1 and increased IGF1-R levels have also been observed due to increased activity of PI3K signaling^{165,166} which increase the nuclear localization of β -catenin and enhanced β -catenin/

Table 2 GSK-3 activity in clinical trials/studies.

Treatment and reference	Outcome	Clinical trial	NCT
The protein kinase C beta inhibitor Enzastaurin results in inhibition of AKT which leads to activation of GSK-3. The effects of Enzastaurin and the vascular endothelial growth factor A (VEGFa) inhibitor Bevacizumab were examined in advanced or metastatic cancer patients. ¹⁵²	Finished Phase I study. 67 patients were evaluable for safety and efficacy. Good results with patients with ovarian cancers. 50.4% of ovarian cancer patients remained without disease progression after 6 months.	Enzastaurin and Bevacizumab in Treating Patients With Locally Advanced or Metastatic Cancer	NCT00550927
To determine effects of combination of enzastaurin and bevacizumab in adults with glioma. ¹⁵³	Finished Phase II study with 81 patients with glioblastomas (GBM, <i>n</i> = 40) and anaplastic gliomas (AG, <i>n</i> = 41). Early response was associated with longer progression free survival for glioblastomas. Combined treatment was well tolerated, and survival time was similar to that observed in patients treated with bevacizumab.	Phase II study with enzastaurin (LY317615) in combination with bevacizumab in adults with recurrent malignant gliomas.	NCT00586508
Combining the EGFR/HER2 inhibitor with the proteasomal inhibitor bortezomib. Lapatinib should inhibit AKT activity which will lead to GSK-3 activity. ¹⁵⁴	Phase I study was terminated due to withdrawal of sponsor support	A Phase I Study of the HER1, HER2 Dual Kinase Inhibitor, Lapatinib Plus the Proteasomal Inhibitor Bortezomib in Patients With Advanced Malignancies	NCT01497626
Effects of Trametinib MEK inhibitor and pan AKT inhibitor (GSK2141795) treatment in melanoma. Suppression of AKT should result in increased. ¹⁵⁵	Completed, Phase II clinical study did not reveal any clinical benefit of trametinib and GSK2141795 treatment in melanoma patients with NRAS mutations or wild-type melanoma. GSK 2141795 inhibited phosphorylation of GSK3 β	Trametinib With GSK2141795 in BRAF Wild-type Melanoma	NCT01941927
Treatment of humans and mouse model of recurrent GBM with temozolomide (TMZ) and other drugs which suppress GSK-3 β (cimetidine, lithium, olanzapine, and valproate, (CLOVA) cocktail. The safety and efficacy of the CLOVA cocktail) in combination with TMZ were performed to human and murine studies. ¹⁵⁶	Inhibition of active GSK-3 β in the tumor resulted in increased patient survival. The combination of TMZ and the CLOVA cocktail significantly inhibited cell invasion and TMZ increased survival compared to patients treated with TMZ alone. Active GSK-3 β was associated with a poor prognosis	Clinical study in Japan completed with 7 GBM patients.	

AR interactions.¹⁶⁷ These findings firmly suggested the PTEN mediated regulation of IGF-1R.¹⁶⁸

Cross-activation

The activation of PI3K signaling axis by the action of GSK3 has also been elucidated in several literatures. Sometimes, the GSK3 β plays an important role in the embryonic angiogenesis of zebrafish through a positive feedback loop of PI3K/Akt and Wnt/ β -catenin signaling cascade. The study has demonstrated that, in *gsk3b*-mRNA-overexpressed embryos there is increased level of phosphorylated GSK3 β , which in turn prevents the proteosomal degradation of β -catenin and thus up regulated the

phosphorylated Akt activity in intersegmental vessels that leads to the up regulation of VegfAa expression.¹⁶⁹

Pathway convergence

The PI3K and GSK3 signaling cascade often acts on same protein to regulate several cellular modalities. A diverse array of proteins have been identified as a substrate of GSK3 which are being phosphorylated by GSK3 and partly regulated by the Akt mediated phosphorylation of GSK3. The RNA binding protein and suppressor of p53 translation, RNPC1 has been identified as an important substrate of GSK3.¹⁷⁰ GSK3 mediated phosphorylation of RNPC1 derepressed the p53 translation stimulated by

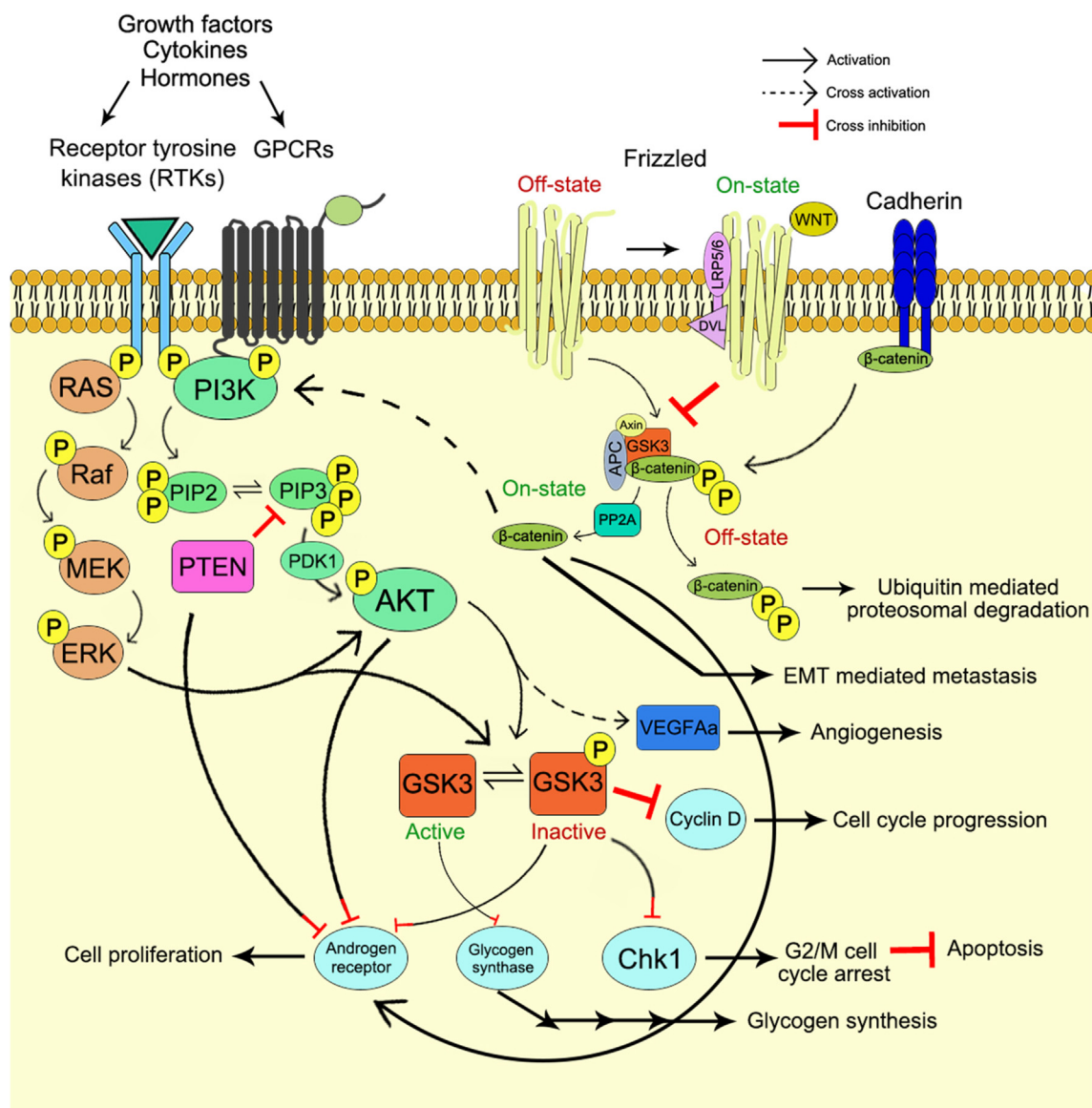


Figure 3 Interplay between PI3K and GSK3 signaling cascade through cross activation and cross inhibition.

PI3K/Akt activity. On the other hand, pharmacological inhibition of Akt showed reduced phosphorylation of GSK3 and increased the RNPC1 phosphorylation causing increased translation of p53. Similarly, there are several proteins recognized as the substrate of PI3K/Akt signaling that also get phosphorylated by the action of GSK3. The Akt activator RICTOR is acted upon by GSK3 through the phosphorylation of TORC2 in a phosphodegron sequence.¹⁷¹ Furthermore, GSK3 α can bind and phosphorylate Akt1 on Thr 312 and leads to the inhibition of Akt kinase activity.¹⁷² PI3K dependent activation of Akt caused phospho-inhibition of TSC2 leads to the downstream activation of mTOR and promote anabolic metabolism. In this instance, GSK3 has been found to mediate the phosphorylation of TSC2 in distinct inhibitory site along with Wnt ligands which promoted the Wnt stimulated mTOR activation.^{104,173} GSK3 β and Akt both involved in a complex interplay of phosphor-regulation and direct

regulation of AR activity. GSK3 β directly phosphorylated the AR hinge and ligand binding regions which leads to the deactivation of AR target genes and triggers cell proliferation.^{162,163} Additionally, Akt has a complex relationship with AR as it preferentially phosphorylated hAR at Ser210 and Ser790^{174,175} which causes both induction¹⁷⁶ and inhibition of AR transactivation.^{174,175}

Effect of GSK3 on the activity of PI3K inhibitors

Involvement of GSK3 in the resistance

Dual PI3K/mTOR inhibitor

It has been frequently observed that the long term exposure of PI3K inhibitors associated with acquired resistance which in turn limits the therapeutic efficacy of the drug. In order to prevent the therapeutic resistance towards the

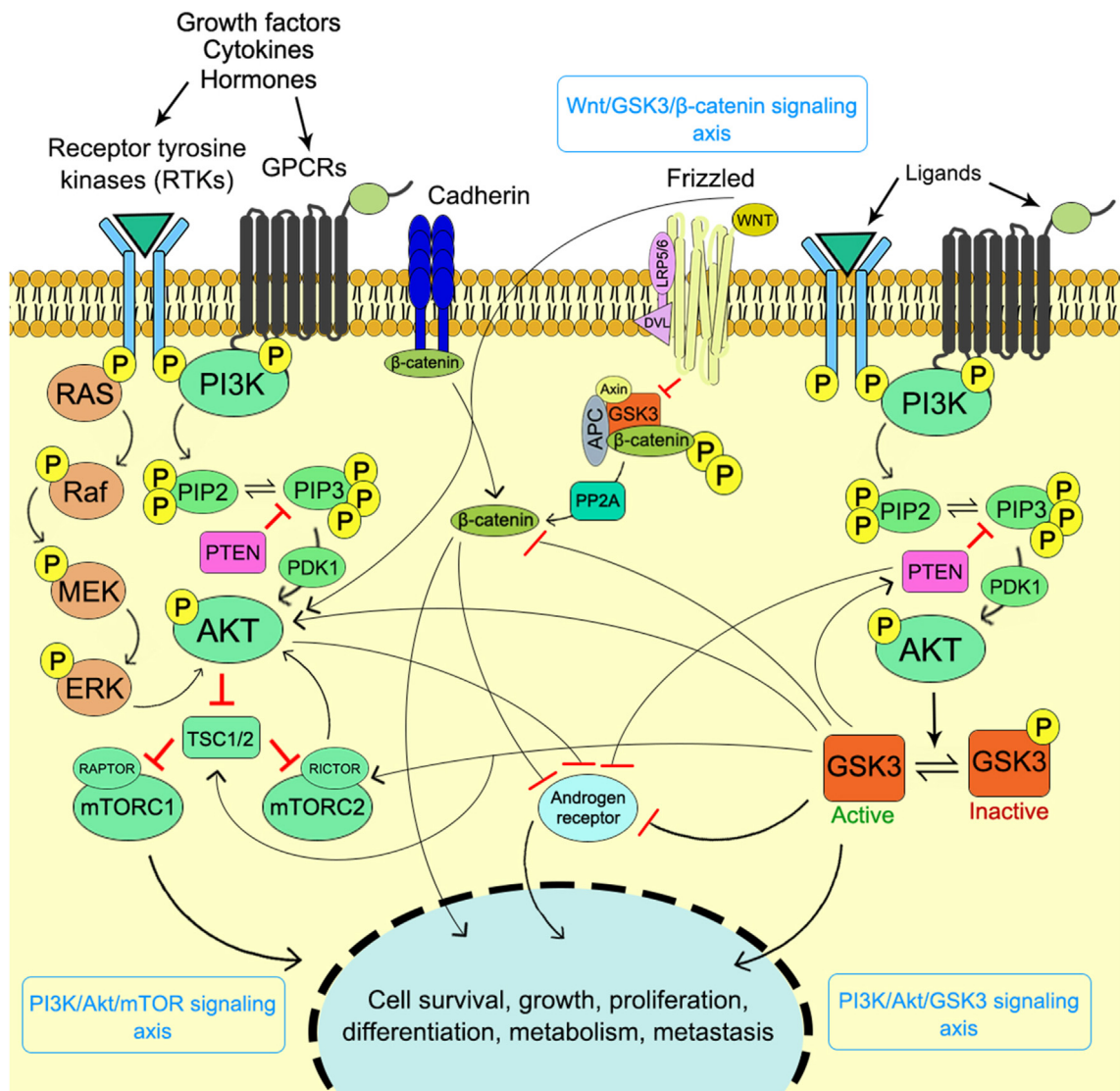


Figure 4 The pathway convergence between PI3K and GSK3 signaling axes.

cancer cells an in depth mechanism of resistance is urgently needed to improve the chemotherapeutic efficacy of the drugs targeting PI3Ks. The detailed mechanism for resistance against the PI3K inhibitors has been showcased in Figure 5. The Wnt/ β -catenin signaling pathway is negatively regulated by the GSK3. The GSK3/Wnt/ β -catenin signaling axis has been found to be involved in the development of mTOR inhibitor resistance in the human colorectal cancer (CRC) cells. The development of resistance against dual PI3K/mTOR inhibitor such as PF-05212384 (gedatolisib) has been demonstrated in all CRC cell lines which is responsible for the frameshift mutation (c.465_466insC; H155 fs*) in T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) 7 (TCF7). In the resistant cell line an increase level of active Tyr216 p-GSK3 β and decrease level of inactive Ser9 p-GSK3 β was observed due to TCF7 frameshift mutation. The active GSK3 β caused increased association of mTOR with Raptor and enhanced the mTORC1 activity in the gedatolisib resistant cells.¹⁷⁷ Hence, the down regulation of active GSK3 β expression through the pharmacological inhibition of

GSK3 β re-established the sensitivity of the resistant cells to gedatolisib via inhibition of mTORC1 activity.¹⁷⁷ Therefore, the combination treatment with GSK3 inhibitor along with the dual PI3K/mTOR inhibitor might be the novel approach in the treatment of resistant cells which is characterized with high expression of active GSK3 β in CRC. In the human primary glioblastoma (GBM) cells the involvement of GSK3 β in the dual PI3K/mTOR inhibitor (NVP-BE2235) resistance has been well documented which is primarily responsible for the chronic exposure of the drug itself.¹⁷⁸ In this instance, the combination treatment with selective GSK3 β inhibitor CHIR99021 resensitizes the resistant cell lines. Moreover, the downregulation of GSK3 β through the shRNA strategy also markedly increases the sensitivity of GBM cells to NVP-BE2235.¹⁷⁸ The knockdown of Rictor, an mTORC2 component, via shRNA prevented the development of resistance to NVP-BE2235, which suggested the involvement of mTORC2. The MEK inhibitor AZD6244 decreases the resistance against NVP-BE2235 which justified the involvement of MEK/ERK signaling cascade. The phosphorylation of

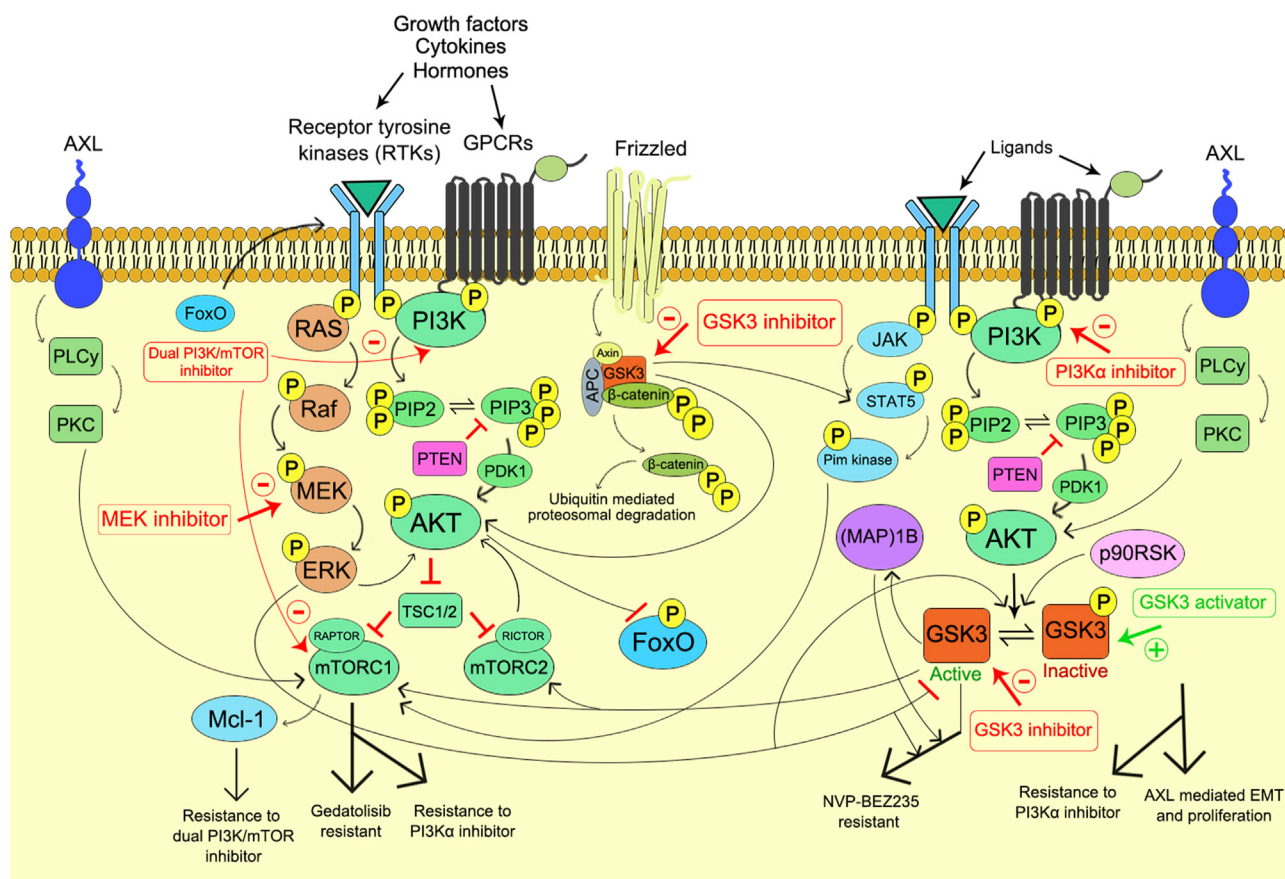


Figure 5 Schematic representation of resistance developed on cancer against PI3K inhibitors through GSK3 modulation.

Akt at Ser473 by mTORC2 is essential for the inhibition of nuclear localization of Forkhead box O (FoxO) protein.⁴ Thus, the long term inhibition of Akt activity by the exposure of NVP-BE2235 leads to increased activity of FoxO protein which upregulated the expression of several receptor tyrosine kinases (RTKs).¹⁷⁹ This enhanced expression of RTKs activated the MEK/ERK signaling cascade.¹⁸⁰ Increased ERK activation caused the phosphorylation of GSK3 β at Thr43 which leads to p90RSK (downstream target of ERK) mediated subsequent phosphorylation of GSK3 β at Ser9, that causes the inactivation of GSK3 β .¹⁸¹ Among various RTKs, the human epidermal growth factor receptor 2 (HER2) and HER3 exhibited significant expression, which play the pivotal role in the pathophysiology of human GBM.¹⁸² The MEK/ERK/p90RSK/GSK3 β signaling axis plays an important role in several human tumors, such as breast, stomach, kidney and liver cancer.¹⁸¹ Further studies have identified the microtubule-associated protein (MAP)1B, a downstream target of MEK/ERK/p90RSK/GSK3 β signaling axis that develops resistance to NVP-BE2235 in human GBM cells. As MAP1B is a downstream target of GSK3 β , the pharmacological inhibition of GSK3 β or the shRNA approach to downregulate the GSK3 β expression caused decreased level of p-MAP1B. Whereas, the exact mechanism for the MAP1B mediated development of resistant against NVP-BE2235 is still unknown. The rapamycin treated GBM cells also developed the GSK3 β /MAP1B dependent drug resistance due to long term exposure of the drug which dampens

the mTORC2 activity in various cancer model systems.¹⁷⁸ These outcomes provides sufficient evidence to outline the mechanism for the development of GSK3 mediated resistance against dual PI3K/mTOR inhibitor which correlates the mTORC2 inhibition and MEK/ERK activation through the MEK/ERK/p90RSK/GSK3 β signaling axis. Additionally, the findings also suggested the GSK3 as a prognostic factor in several cancer model systems which act in the other way to enhance the patient outcome.⁸⁵ Thus, the pharmacological inhibition of GSK3 β may attribute to the combinations therapy in the resistant cell lines while it may also negatively impact the other aspects of GBM pathophysiology. The development of PI3K/Akt inhibitor resistance in the cancer cells is also associated with the increased expression of Pim kinases through the activation of STAT5 which enhanced the mTORC1/Mcl-1 pathway.¹⁸³ The dual PI3K/mTOR inhibitor triggered a positive feedback loop to increase the activation of JAK2/STAT5 and IL-8 secretion which contributes to the drug resistance.¹⁸⁴ Moreover, the GSK3 β also contributes to the activation of STAT5 through the tyrosine phosphorylation at Tyr694 by either IFN α or IFN γ ,^{185,186} whereas the GSK3 α responsible for different regulatory activities. The combination therapy of GSK3 β inhibitor with dual PI3K/mTOR inhibitor might result in sensitization of resistant cells towards the PI3K inhibitor through the downregulation of STAT5 activation which leads to the inhibition of STAT5 mediated mTORC1/Mcl-1 pathway.

Selective PI3K isoform inhibitors

Other mechanisms of resistance development include the activation of AXL. In the head, neck and oesophageal squamous cell carcinomas (OSCC) the resistance against PI3K α develops through the activation of receptor tyrosine kinase AXL which promotes resistance via induction of phospholipase C (PLC γ) and protein kinase C (PKC) and results in PI3K independent mTOR activation.¹⁸⁷ The sensitivity towards the PI3K α inhibitor could be restored through the repression of AXL.¹⁸⁸ Additionally, the activation of AXL is associated with the epithelial–mesenchymal transition (EMT) which further leads to oncogenic metastasis of the cancer cells.^{189,190} In this instance, the repression of AXL expression caused decreased proliferation, invasion and migration of OSCC cells. Furthermore, the repression of AXL decreased the Akt activation and subsequent inhibition of NF- κ B transcriptional activity. The inactivation of Akt further activates the GSK3 β which in turn results in the inhibition of EMT through the upregulation of epithelial markers. Thus, it can be hypothesized that the induction of GSK3 β activity decreased the AXL mediated EMT and proliferation of cancer cells and sensitizes the cancer cells towards PI3K α inhibitor.¹⁹¹

Conclusion

In various cancer model systems, the abnormal activation of PI3K signaling pathway is the major cause for the initiation and progression of malignancy with therapeutic resistance towards the chemotherapeutic management due to long term exposure of the drugs. The development of the resistance against the PI3K inhibitor has become the major concern in the treatment of cancer which is associated with the treatment failure and cancer relapse as observed in various cancer incidences. Therefore, the elimination of the resistance towards PI3K inhibitors through in depth understanding of the mechanism for specific resistance is the primary objective in the field of cancer chemotherapy.

The function of GSK3 in cancer is very conflicting and ambiguous. GSK3 involves in several signaling pathways through complicated regulatory networks which directly contributes to cancer initiation and differentiation. However, the dual role of GSK3 in cancer demonstrated its function as either cancer promoter or inhibitor as per the cell type.

Indeed, the PI3K and GSK3 signaling axis are interconnected through a complex regulatory downstream cascade which plays the key role in several malignancies via the manipulation of cell survival strategies which includes disruption of apoptosis, increased angiogenesis, EMT mediated metastasis. In various clinical settings, the up-regulated activity of GSK3 plays the pivotal role in the development of therapeutic resistance against PI3K inhibitor. Therefore, it can be hypothesized that the combination therapy of GSK3 inhibitor with PI3K inhibitor might be a significant approach to sensitize the cancer cells to chemotherapeutic treatment by suppressing the GSK3 mediated drug resistance. On the other hand, in some other cancer phenotypes the activation of GSK3 is directly related to the suppression of drug resistance and for those

incidences the specific GSK3 activators possessed the vital role to elevate the function of PI3K inhibitor through their sensitization towards the target cells. However, the development of such specific GSK3 activators has not been reported till date.

The paradoxical activity of GSK3 in various cancer cell types abolished the development of the targeted GSK3 therapy in cancer chemotherapy. Although, extensive investigation on the mechanism of GSK3 is readily required to unravel the relation between the activation of GSK3 with the cancer prevention through the PI3K inhibition and sensitization of cancer cells against the PI3K inhibitor. Besides, this approach will also deliver an insight into the components of GSK3 signaling axis which might be further targeted for therapeutic interventions.

In conclusion, the role of specific GSK3 activator or inhibitor subjected to cancer phenotypes might be a significant approach which not only improves the activity of PI3K inhibitor but also targets several GSK3 substrates to unleash new treatment modalities against neoplastic transformation.

Author contributions

Abhijit Das: Article writing, processing of illustration, research design; Barshana Bhattacharya: Data collection, article writing, conceptualization; Souvik Roy: Article revision, final approval.

Conflict of interests

Authors declare no conflict of interests.

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