Recent Patents Related to Phosphorylation Signaling Pathway on Cancer

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Received: June 13, 2011 Revised: July 04, 2011 Accepted: July 23, 2011

Abstract: Phosphorylation and dephosphorylation play an important role in the regulation of growth factor and cytokine signal transduction to modulate cell proliferation, differentiation, survival, and apoptosis. In some cellular systems, the information suggests that EGFR, somatostatin receptors, SHP-1, Akt and PI3K can regulate carcinogenesis implied process through regulated the activity of NF- B. Current patents related to signaling pathway that includes somatostatin receptors, phosphotyrosine phosphatases, tyrosine kinases, AKT/PKB and PI3K are focusing in diagnosis, prognosis and treatment. Many recent patented techniques include inhibition, antagonism or alternative therapeutic methods. Furthermore, it is necessary to deepen understanding of the molecular mechanisms involved in cancer to develop other alternative therapies focusing not only on new inhibitors.

Keywords: Phosphotyrosine kinases, phosphotyrosine phosphatases, signaling pathway, diagnosis, treatment, cancer.

1. INTRODUCTION

The activation of proteins by post-translational modification represents an important cellular mechanism for regulating most aspects of biological organization and control, including growth, development, homeostasis and cellular communication. Protein phosphorylation plays a critical role in the etiology of many pathological conditions and diseases, including cancer, developmental disorders, autoimmune diseases and diabetes. In spite of the importance of protein modification, it is not yet well understood at the molecular level. The reasons for this lack of understanding are, first, that the cellular modification system is extraordinarily complex, and second, that the technology necessary to unravel its complexity has not yet been fully developed [1]. The complexity of protein modification includes phosphorylation and dephosphorylation on proteins of different signaling pathways corresponding to growth, development, disease states and aging [2]. The human genome encodes many kinases and phosphatases families, making them the most abundant class of enzymes known [3].

Protein tyrosine phosphorylation is regulated in the cell by the opposing activities of two enzymes: protein tyrosine kinases (PTKs), which transfer phosphate from ATP to substrate proteins, and protein tyrosine phosphatases (PTPs), which remove it [4]. Intercellular protein tyrosine phosphorylation is regulated by extracellular stimuli, such as cytokines, to control cell growth, differentiation and functional activities. This signaling mechanism depends on the interplay of protein tyrosine kinases, which initiate signaling cascades through phosphorylating tyrosine residues in protein substrates, and by protein tyrosine phosphatases that terminate signaling via substrate dephosphorylation [5].

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Phosphoprotein phosphatases can be generally classified into four major groups based on their arrangement on the cell membrane and their substrate utilization: receptor classic phosphotyrosine phosphatases, non receptor classic phosphotyrosine phosphatase, dual – specifity phosphatases which dephosphorylate substrates on tyrosine, serine and/or threonine residues and lipids phosphotyrosine phosphatases [6] (Table **1**).

Kinases can be classified into protein kinases and lipid kinases, and certain kinases exhibit dual specificities. Protein kinases can be generally classified into three major groups based upon their substrate utilization: tyrosine kinases that predominantly phosphorylate substrates on tyrosine residues (eg, erb2, PDGF receptor, EGF receptor, VEGF receptor, src, abl) serine/threonine kinases that predominantly phosphorylate substrates on serine and/or threonine residues (eg. mTorC1, mTorC2, ATM, ATR, DNA-PK, Akt) and dual – specifity kinases that phosphorylate substrates on tyrosine, serine and/or threonine residues [7].

Each of these kinases phosphorylates specific serine, threonine, or tyrosine residues located within distinct amino acid sequences, or motifs, contained within different protein substrates. Most kinases phosphorylate many different proteins, and many proteins are phosphorylated at multiple sites by different kinases [8].

Phosphorylation is important in signal transduction mediated by receptors via extracellular biological signals such as growth factors or hormones. For example, many oncogenes are kinases or phosphatases. In addition, a kinase or phosphatase can have its activity regulated by one or more distinct kinases or phosphatases, resulting in specific signaling cascades. The ability to identify phosphorylation sites on a wide variety of cellular proteins, and kinases and phosphatases involucrate in these processes, is crucially important to understanding the key signaling proteins and pathways implicated in cancer progression [9].

1872-2156/11 $100.00+.00

Table 1. Families of PTPs and Relation with Function [6]

Type of PTP	Function	Examples
Receptor Classic PTP	Signaling pathways of immunological response	LAR, CDC45
Non Receptor Classic PTP	Signaling pathways of immunological response, proliferation, cell adhesion and differentiation.	SHP-1 (PTPN6), SHP-2 (PTPN11), PTP1B, PEST.
Duals PTP	Signaling pathways of differentiation, cellular cycling and apoptosis.	CDC25, MKP, VHR
Lipids Phosphotyrosine Phosphatases.	Signaling pathway of cellular surviving.	PTEN

The PI3K/Akt pathway mediates the effects of a variety of extracellular signals in a number of cellular processes including cell growth, proliferation, and survival. Deregulation of the PI3K/Akt pathway in many cancers may be implicated in tumorigenesis. A more comprehensive understanding of the signaling intricacies is necessary to develop clinical applications of the modulation of this pathway in this pathology. The intervention on this pathway through inhibitors or antagonist increases the efficiency of therapies and can thus lead to cancer control. The signaling cascade is initiated when the activation of a tyrosine kinase receptor, such as epidermal growth factor receptor-1 and insulin-like growth factor receptor, occurs. In addition, some phosphotyrosine phosphatases, such as SHP-1, or the activation of antiproliferative receptors, such as somatostatin, can also regulate this pathway. Understanding which proteins are modified by these signaling pathways will greatly expand our possibilities of the development new therapeutic alternatives [10].

The present review is an updated version of previous paper that summarizes important findings from recent patent of phophotyrosine phosphatases SHP-1 and includes recently development kinases inhibitors targeting the related pathways PI3K / Akt and SSTR2 / SHP-1.

2. SSTR2 / SHP-1 SIGNALING PATHWAY

Previously data have demonstrated SSTR2, Gi and SHP-1 co-immunoprecipitate *in vitro* and *in vivo* [39]. SSTR2 is a G protein coupled receptor that regulates downstream SHP-1 activity in the antiproliferative effect of epithelial cells [40].

2.1. Recent Patent Related Phosphotyrosine Phosphatases SHP-1

Protein tyrosine phosphatases (PTPs) constitute a large family of signaling enzymes (>100 in humans) that are important for the regulation of cell proliferation, differentiation, metabolism, migration and survival [11]. Dysfunction in PTPs results in aberrant tyrosine phosphorylation, which has been linked to the etiology of several human diseases, including cancer and diabetes [12].

Unlike protein kinases, where tyrosine specific and serine/threonine specific kinases share sequence identity, the PTPs show no sequence similarity with serine/threonine phosphatases, or the broad specificity phosphatases such as acid or alkaline phosphatases. The hallmark that defines the PTP superfamily is the active site amino acid sequence $C(X)_5R$, also called the PTP signature motif, in the catalytic domain. This motif is conserved among all members of the PTP superfamily. The PTPs can be broadly divided into two groups based on active site substrate specificity [6, 13].

Although PTPs share a common catalytic mechanism, they have distinct (and often unique) biological functions *in vivo*. One of the major challenges in the field is to rapidly establish the functional roles for PTPs, in both normal physiology and pathogenic conditions. Gene knockout analysis is useful to assess the role of a number of PTPs in cellular signaling. However, this process is often tedious, and gene ablation in animals often results in compensatory changes through other mechanisms during embryonic development [14]. One attractive strategy for efficient analysis of PTP function is to characterize these enzymes collectively, rather than individually [15]. In this regard, DNA microarray methods provide significant insights into changes in the abundance of transcripts [16].

Cloning procedures aided by homology searches of expressed sequence tag (EST) databases have accelerated the pace of discovery of new genes, but EST database searching remains an involved and onerous task. More than 3.6 million human EST sequences have been deposited in public databases, making it difficult to identify ESTs that represent new genes [17].

The analysis of gene expression within cells has been used to provide information on the state of those cells and importantly the state of the individual from which the cells are derived. The relative expression of various genes in a cell has been identified as reflecting a particular state within a body [18]. For example, cancer cells are known to exhibit altered expression of various proteins and the transcripts or the expressed proteins may therefore be used as markers of that disease state. The identification of quick and easy methods of sample analysis for diagnostic applications remains the goal of many researchers [19]. End users seek methods which are cost effective, produce statistically significant results and which may be implemented routinely without the need for highly skilled individuals.

However, the measured mRNA levels do not always correlate with protein expression. Proteomic technologies are appropriate for measurement protein levels to complement genomic and transcriptómica data. Unfortunately, current

methodologies are only adequate for abundant proteins. Furthermore, the amount of protein is not always proportional to biological activity, which may subject to post-translational regulation. Because the function of a PTP depends on its phosphatase activity, the development of novel technologies for directly measuring the dynamics in PTP activity on a global scale is of tremendous interest [20].

Functionally, tyrosine phosphorylations generate high affinity binding sites for other proteins containing SH2 domains. SHP-1 (PTPN6) is one of the most important components for phosphorylation equilibrium. Decreased activity could be cause pathological consequences. [4] It has now been found that two PTP constitutive non receptor protein tyrosine phosphatases, SHP-1 and SHP-2, play a role in the negative regulation of BCR-ABL and that lack of SHP1 may be important for CML transformation. SHP-1 and SHP-2 are two SH2 domain containing tyrosine phosphatases with several pathological implications on cell growth regulating signaling. They share significant overall sequence identity [21].

For the success of treatment with imatinib mesylate in chronic myeloid leukemia (CML) patients requires a measured of critical biomarkers of treatment response. **WO 2010054045 (A1)** relates to a method for evaluating patients to help optimizing the treatment of CML in a human patient population. The present invention proposes the use of SHP-1 and/or SHP-2 as a biomarker for monitoring imatinib mesylate treatment. The level of SHP-1 and/or SHP-2 is indicative for the therapeutic efficacy of imatinib or a pharmaceutically acceptable salt thereof. The technique includes an *ex vivo* method for determining the SHP-1 and/or SHP-2 level, comprising the determination of SHP-1 and/or SHP-2 mRNA levels from a sample; mRNA level of ABL and normalization of SHP-1 and/or SHP2 mRNA to ABL [22].

SHP-2, encoded by the PTPN11 gene, is a non-receptor PTP containing two SH2 domains, a PTP domain, and a C-terminal region. It is part of the SH2 domain necessary for embryonic development and growth factor, cytokine, and extra-cellular matrix signaling and effect cell proliferation, differentiation, and migration. The N-SH2 domain in the wild type SHP2 interacts with the PTP domain, resulting in autoinhibition of the SHP-2 PTP activity [23].

A well recognized SHP-2 regulated signaling pathway is the Ras-Erk1/2 MAP pathway. For instance, SHP-2 is positively involved in EGF stimulated Erk1/2 activation. SHP-2 PTP activity is required for transformation of human glioblastoma cells by EGFRv III and human mammary epithelial cells by ErbB2 [30]. In the last few years, mutations in the SHP-2 gene PTPN11 have been identified in several types of leukemias and in some cases of solid tumors [24].

Mutations in the PTPN11 gene and SHP-2 can cause Noonan syndrome, juvenile myelomonocytic leukemia, acute myelogenous leukemia and LEOPARD (lentigines, electrocardiogram abnormalities, ocular hypertelorism, pulmonic valvular stenosis, abnormalities of genitalia, retardation of growth, and deafness). Within these diseases, SHP-2 is uninhibited and interacts with the docking protein Gab family. This interaction activates a pathway leading to cell proliferation and tumorigenesis. The identification of SHP-2 role in these diseases is very important for developing cancer therapy. Targeting and inhibiting SHP-2 with small molecule inhibitors has become a major goal in developing a new cancer therapy. These mutations and other cancer associated SHP-2 mutants are predicted or have been demonstrated to be gain of function mutations. Importantly, no loss of function SHP-2 mutant has ever been found in human cancer [25].

Several reports indicated that SHP-2 is a negative regulator of interferon (IFN) signaling. SHP-2 was able to dephosphorylate STAT1 *in vitro*, suggesting that STAT1 is a substrate of SHP-2 PTP. Consistently, increased IFN-stimulated STAT1 tyrosine phosphorylation was observed in mouse embryonic fibroblast cells lacking a functional SHP-2. The inhibitory effect of SHP-2 on STAT1 tyrosine phosphorylation may contribute to modulation of the antiviral effect of IFN. While PTPs have increasingly attracted attention as novel targets of cancer drug discovery, only a few selective PTP inhibitors have been characterized biologically [26].

A high throughput screen identified compounds that selectively inhibit SHP-2. Developing a SHP-2 specific inhibitor is complicated by the similarity between SHP-1 and SHP-2, which share 60% overall sequence identity and approximately 75% similarity in their PTP domains. However, SHP-1 and SHP-2 catalytic domains have different substrate specificity, suggesting that the catalytic cleft is not identical [27]. Furthermore, the surface electrostatic potential of the catalytic cleft is much more positive in human SHP-2 than in human SHP-1. Although amino acid residues present at the base of SHP-1 and SHP-2 PTP catalytic clefts are identical, all four sides of the catalytic cleft contain one or more residues that are different between SHP-1 and SHP-2 [28]. In this sense, **WO 2010121212 (A2)** comprises novel SHP-2 inhibitors, a method to develop new SHP-2 inhibitors, and a method of treating cancer using the inhibitors. The development of a SHP-2 specific inhibitor that does not cross-inhibit SHP-1 is important for development of effective treatment modalities [29]. **WO 2009135000 (A3)** relates to cancer therapy. More specifically, this invention relates to compounds useful in specific inhibiting SHP-2 protein tyrosine phosphatase activity [30].

2.2. Recent Patents Related to Somatostatin Receptor 2

Somatostatin receptors are expressed particularly in neuroendocrine cells and in the gastrointestinal tract. Most human tumors originating from the somatostatin target tissue have conserved somatostatin receptors. The somatostatin receptors in tumors can be identified, for example, using *in vitro* binding methods or using *in vivo* imaging techniques; the latter allow the precise localization of the tumors and their metastasis in the patients. Because somatostatin receptors in gastroenteropancreatic tumors are functional, their identification can be used to assess the therapeutic efficacy of an analog to inhibit excessive hormone release in the patients. In light of their use as diagnostic and therapeutic targets, there is a need for somatostatin peptide antagonists that bind strongly to SSTR2, while at the same time showing only minimal propensity for binding to the other four receptors. For use as diagnostic imaging agents, such antagonists would have an advantage over SSTR2 selective agonists in

that the antagonists would preferably not be internalized [31-33].

In this sense, **WO 2011057471 (A1)** relates a family of compounds (verapamil and their stereoisomers) that may bind to endogenous neurotransmiter receptors. These compounds are identified and formulated into a pharmaceutical composition for treating a disease or condition related to orexin receptor 1, orexin receptor 2, somatostatin receptor 2 or dopamine D2L receptor. Verapamil is capable of binding to somatostatin receptor 2, among others [34]. Other example is patent **WO 2009129311 (A3)** that described somatostatin analogs agonists selective for SSTR2 and SSTR5. Related compounds include antagonists complexes with or conjugated to radioactive nuclides and uses thereof. The antagonists of the invention are useful in diagnosing and treating neoplastic and non-neoplastic mammalian diseases [35].

On the other hand, some inventions are related to genomic therapy, such as **US 2010222418 (A1)** that relates to the field of cancer, and in particular to new products, compositions, plasmid vector and methods for cancer therapy. The application is preferably on metastatic cancer, like the pancreatic cancer and hepatocellular carcinoma, and most preferably an exocrine pancreatic cancer. Metastasis corresponds to the process by which a cancer spreads from the place at which it first arose as a primary tumor to distant locations in the body. Since this process is very particular in cancer progression, it is generally necessary to use specific regimen in order to inhibit metastazing. The authors have established that the combined intratumoral injection of an expression vector coding for SSTR2, deoxycytidine kinase protein (DCK) and uridine monophosphate kinase protein (UMK) associated with a gemcitabine administration results in an extensive and surprising decrease of metastasis sites. Accordingly, and in a preferred embodiment, the present invention is directed to the inhibition of tumor spread out, for example the inhibition of tumor metastazing. The "gene expression sequence" is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which facilitates the efficient transcription and translation of the nucleic acid to which it is operatively linked. The promoters useful as gene expression sequences of the invention also include inducible promoters. Inducible promoters are expressed in the presence of an inducing agent. According to another specific embodiment, gemcitabine is administrated by intravenous route [36].

3. RECENT INHIBITORS TO KINASES BLOCKING SIGNALING PATHWAY

Chemical compounds that modulate the activity of protein tyrosine kinases or phosphatases can be of value as experimental tools or as potent therapeutic reagents [37].

The usage of knowledge on molecular pathway signaling can guide new diagnostics methods, prevention and treatment of cancer, especially on blocking kinases implicated on pathway signaling. Table **2** summarized the most relevant patent respect this thematic since 2010.

Defects in various components of signal transduction pathways have been found to account for a vast number of diseases, including numerous forms of cancer, inflammatory disorders, metabolic disorders, vascular and neuronal diseases [12]. Patent **US 20100190749 A1** provides chemical entities or compounds and pharmaceutical compositions, therapeutic applications, thereof that are capable of modulating certain protein kinases such as mTor, tyrosine kinases, and /or lipid kinases such as PI3 kinase. The authors showed the results of cell proliferation inhibition assays performed with a wide range of neoplastic cell lines *in vitro* using conventional anti-cancer drugs. Through western blot and others techniques they demonstrated that the subject mTor inhibitor of the invention is more effective in inhibiting Akt phosphorylation and PI3K pathway activation as compared to rapamycin [38].

Similarly patent **US 7,691,858 B2** is based on the discovery that certain chemical compounds are effective in inhibiting phosphoinositide 3-kinases, most specifically the PI3K / isoforms. Authors claims a pharmaceutical composition selected from the group consisting of 3-(2,4-diaminopteridin-6-y1) phenol and 6-(1H-indol-4-y1)pteridine-2,4-diamine, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier thereof [39].

US 20100190816 A1 relates to compounds that may be used to inhibit kinases as well as compositions of matter and kits comprising these compounds. The present invention relates to inhibitors of enzymes that catalyze phosphate transfer and/or that bin ATP/GTP nucleotides, compositions comprising the inhibitors. The inhibitors and compositions comprising then are useful for treating or modulating disease in which phosphoryl transferases, including kinases, may be involved, symptoms of such disease. Aurora kinases (Aurora –A, Aurora – B, Aurora – C) are serine/threonine protein kinases that have been implicated in human/cancer, such as colon, breast and other solid tumors [40].

Small – molecule inhibitors of the Abelson tyrosine kinase (ABL) and the epidermal growth factor receptor (EGFR) have been developed into clinically useful anticancer drugs. Patent **US 20100256171 A1** relates generally to the inhibition of protein kinases, and includes inhibitors that specifically target certain protein kinases, as well as the engineering or modification of proteins so as to be susceptible to inhibition by such inhibitors [41]. The author of this patent claims a method for inhibiting the proliferation of a tumor cell comprising contacting the cell with an inhibitory – effective amount of a compound having a heterocyclic core structure comprised of two or more fused rings containing at least one nitrogen ring atom, and an electrophilic substituent that is capable of forming a covalent bond with a cysteine residue within the ATP binding site of a kinase. This invention includes small molecule inhibitors of Rsk kinases activity. Rsk – family kinases have been shown to prevent apoptosis in melanoma cells and leukemia cells [42-43]. The Rsk serine/threonine protein kinases have critical functions in the Ras/MAP kinase signaling pathway, a pathway which is deregulated in many human cancers. Inhibition of Rsk in these cancer cell lines by introduction of plasmids encoding dominant interfering mutants of Rsk1 or Rsk2 causes apoptosis, also known as programmed cell death. Of the four Rsk isoforms (Rsk 1-4), Rsk 1 and Rsk2 are directly activated by the MAP kinases, ERK1 and ERK2. Known substrates of Rsk 1,2 include transcription factors involved in cell growth and

Table 2. Recent Patents on Inhibitors to Kinases Blocking Signaling Pathway

Publication Number	Title	Inventors
US 20100190749 A1	Benzoxazole kinase inhibitors and methods of use	Pingda *et al.*, 2010.
US 7,691,858 B2	Kinase inhibitors and methods of use thereof	Doukas *et al.*, 2010.
US 20100190816 A1	Kinase inhibitors	Dong *et al.*, 2010.
US 20100256171 A1	Selective serine/threonine kinase inhibitors	Taunton *et al.*, 2010.
US 20100260677 A1	Methods and systems for treatment and/or diagnosis	Bhatia and von Maltzahn, 2010.

differentiation (e.g. CRB, c-fos, estrogen receptor) and apoptosis (NF-kB) [44].

Other patents include endothelial cell markers or tumor markers. Patent **US 20100260677 A1** relates to methods and systems that can be used for treatment and/or diagnosis. Involves systems and methods for activating a biological cascade in a subject, and administering, to the subject, a composition or a component comprising an agent able to bind a product of a biological cascade or otherwise interact with the biological cascade. The biological cascade may be, for example, a coagulation cascade, a complement cascade, an inflammation cascade, or the like. By activating the biological cascade, e.g., with an activation composition or by applying energy, coagulation may be induced in a tumor, which the antitumor species may associate with due to an increase in fibrin caused by the coagulation cascade. The targeting entity is associated with a specific developmental stage or a specific disease stat, i.e. a marker. Numerous markers are known in the bibliography. Typical markers include cell surface proteins, e.g. receptors. Examples of receptors include, but are not limited to, the transferring receptor, LDL receptor, growth factor receptors such as epidermal growth factor receptor family members (e.g. EGFR, HER -2, HER-3, HER -4, HER -2/neu, etc.) or vascular endothelial growth factor receptors, cytokine receptors, cell adhesion molecules, integrins, selectins, CD molecules, etc. the marker can be, in some cases, a molecule that is present exclusively or in higher amounts on a malignant cell, e.g., a tumor cell, relative to normal tissue. For example, prostate-specific membrane antigen (PSMA) is often expressed at the surface of prostate cancer cells [45].

4. PI3K / AKT (PKB) SIGNALING PATHWAY

The **PI3K / Akt (PKB)** pathway appears important for regulating cell survival/cell death. Survival factors, such as platelet derived growth factor (PDGF), nerve growth factor (NGF) and insulin-like growth factor-1 (IGF-I), promote cell survival under various conditions by inducing the activity of PI3K. Activated PI3K leads to the production of phosphatidylinositol (3,4,5)-triphosphate (IP3), which in turn binds to, and promotes the activation of, the serine/threonine kinase Akt, which contains a pleckstrin homology domain (PH) [46].

4.1. Recent Patents Related to Akt (PKB)

Analysis of Akt levels in human tumors showed that Akt2 is overexpressed in a significant number of ovarian and pancreatic cancers. Similarly, Akt3 was found to be overexpressed in breast and prostate cancer cell lines. It was demonstrated that Akt-2 was over-expressed in 12% of ovarian carcinomas and that amplification of Akt was especially frequent in 50% of undifferentiated tumors, suggestion that Akt may also be associated with tumor aggressiveness. Increased Akt1 kinase activity has been reported in breast, ovarian and prostate cancers [47].

Some researchers around the world patented a novel compounds that acts as PKB / AKT inhibitors in the treatment of cancer and arthritis. Table **3** summarized novel patent 2010 - 2011 [48-63].

Inhibitors of Akt are useful in the treatment of tumors, especially those with activated Akt. PTEN is a critical negative regulator of Akt and its function is lost in many cancers, including breast and prostate carcinomas, glioblastomas, and several cancer syndromes. Inhibition of Akt has also been implicated in the treatment of leukemias. Akt3 is up-regulated in estrogen receptor-deficient breast cancers and androgen-independent prostate cancer cell lines and Akt2 is over-expressed in pancreatic and ovarian carcinomas. Akt1 is amplified in gastric cancers and up-regulated in breast cancers. Therefore a small molecule Akt inhibitor is expected to be useful for the treatment of these types of cancer as well as other types of cancer. Akt inhibitors are also useful in combination with further chemotherapeutic agents [47].

Some patents such as **WO 2010101734 (A1)** include the combination anticancer therapy with certain Akt inhibitor and other anticancer agents such as anticancer antimetabolites, anticancer antibiotics, plant-derived anticancer agents, anticancer platinum-coordinated complex compounds, anticancer camptothecin derivatives and anticancer tyrosine kinase inhibitors [64].

WO 2011009059 (A2) disclosed is a method for inhibiting Akt, activating AMPK, inhibiting mTOR, treating or preventing development of cancer, and/or preventing progression of premalignant lesions to cancer. The method involves the use of these compounds as single agents or in combination with conventional chemotherapy, biological therapy, or radiation therapy. The invention further provides novel compounds and pharmaceutical compositions for use in treating or preventing development of cancer or preventing the progression of premalignant lesions into cancer. Also disclosed is a method of inhibiting CSF1R [65].

Table 3. Novel Compounds Patents that Acts as PKB / AKT Inhibitor

Patent Number	Type of Inhibitor	Inventors
US 2011098221(A1)	Novel hetero-pyrrole compounds inhibitors of AKT activity	Lin *et al.*, 2010
WO 2011055115(A1)	Novel hetero-pyrrole compounds inhibitors of AKT activity	Bell *et al.*, 2011
US 2011092423(A1)	Novel hetero-pyrrole compounds inhibitors of AKT activity	Rouse and Seefeld, 2011
US 2011092511(A1)	Novel substituted naphthyridine compounds that inhibit Akt activity.	Furuyama *et al.*, 2011
US 2011071182(A1)	Novel heterocyclic carboxamide compounds that inhibit Akt activity.	Seefeld *et al.*, 2011
US 2011065716(A1)	Novel hydroxylated pyrimidyl cyclopentanes that inhibit Akt activity.	Bencsik *et al.*, 2011
US 2011053959(A1)	Novel pyrimidyl cyclopentanes that inhibit Akt activity.	Banka *et al.*, 2011
US 2011015242(A1)	Novel hetero-pyrrole compounds inhibitors of AKT activity	Chen Ching, 2011
MX 2010007546(A)	Novel hydroxylated pyrimidyl cyclopentane that inhibits Akt activity.	Liang *et al.*, 2010
WO 2010114780(A1)	Novel substituted fused pyrimidine compounds that inhibit Akt activity.	Chen *et al.*, 2010
WO 2010104933(A1)	Novel substituted fused naphthyridine derivatives that inhibit Akt activity.	Fan *et al.*, 2010
WO 2010104705(A1)	Novel substituted fused naphthyridine derivatives that inhibit Akt activity.	Sanderson *et al.*, 2010
MX 2010001745(A)	Fused imidazoles for cancer treatment.	Maier *et al.*, 2010
WO 2010088177(A1)	Novel hetero-pyrrole compounds inhibitors of AKT activity	Armstrong *et al.*, 2010
US 2010075970(A1)	Novel compounds which contain substituted pyridazines and pyrimidines moieties which inhibit the activity of AKT.	Bilodeau *et al.*, 2010
US 2010168123(A1)	The present invention provides compounds with the formula: A-L-CR where CR is a cyclical core group, L is a linking group and A is as defined herein.	Mitchell *et al.*, 2010

WO 2010075443 (A1) describes an improved method for screening compounds for activity in inhibiting the enzymatic activity of Aktl protein kinase an enzyme that is believed to play a key role in the inhibition of apoptosis and thus in the etiology of cancer and other conditions, including neurodegenerative diseases. Methods for identifying a cancer patient, such as a breast cancer patient, suitable for treatment with a 4-anilinoquinazoline kinase inhibitor, such as lapatinib, and an AKT inhibitor, comprising detecting modulated expression of HER2 (ERBB2) and SASH1 or protein encoded thereof and detecting PIK3CA mutation status. High levels of expression in HER2 and high levels of SASH1 and/or positive PIK3CA mutation status indicate a patient that is suitable for treatment with a 4-anilinoquinazoline kinase inhibitor, such as lapatinib and an AKT inhibitor [66].

WO 2011060380 (A1) relates generally to genetic markers involved in the diagnosis and prognosis of ERBB2 / HER2 -positive cancer (HER2 positive), predicting patient response to specific therapeutic compounds and providing such therapy to patients predicted to benefit from such therapy [67].

Some patents provides a cancer biomarker based on detection phosphorylation events of AKT on Tyr 176 (**WO 2010091354 A2) and** Ser473 (**WO 2010135671 A1**) [68-69].

The patent **US 2010092473 (A1)** relates to antibodies that immunospecifically bind to phospho-Akt and certain p-Akt substrates. The invention encompasses human and humanized forms of the antibodies and their use in treating cancers and other proliferative disorders. The invention also relates to p-Akt-derived peptides useful for preparing the antibodies. Methods and compositions for detecting, diagnosing, treating or ameliorating a disease or disorder, especially cancer and other proliferative disorders using the present antibodies also are disclosed [70].

4.2. Patents Related to PI3K

The phosphoinositide-3-kinases (**PI3K**) are a family of enzymes that regulate diverse biological functions in every cell type by generating phosphoinositide second messenger molecules. PI3K phosphorylate lipids at the 3-hydroxyl residue of an inositol ring to generate phosphorylated phospholipids (PIP3s) which act as second messengers recruiting kinases with lipid binding domains. Binding of Akt to membrane PIP3s causes the translocation of Akt to the plasma membrane, bringing Akt into contact with PDK1, which is responsible for activating Akt. The tumor suppressor phosphatase, PTEN, dephosphorylates PIPs and therefore acts as a negative regulator of Akt activation. The PI3K, Akt and PDK1 are important in the regulation of many cellular processes including cell cycle regulation, proliferation, survival, apoptosis and motility and are significant components of the molecular mechanisms of diseases such as cancer, diabetes and immune inflammation [71].

Several components of the PI3K / Akt / PTEN pathway are implicated in oncogenesis. In addition to growth factor receptor tyrosine kinases, integrin-dependent cell adhesion and G-protein coupled receptors activate PI3K both directly and indirectly through adaptor molecules. Functional loss of

PTEN (the most commonly mutated tumor-suppressor gene in cancer after p53), oncogene mutations in PI3K, amplification of PI3K and overexpression of Akt have been established in many malignancies. In addition, persistent signaling through the PI3K / Akt pathway by stimulation of the insulin-like growth factor receptor is a mechanism of resistance to epidermal growth factor receptor inhibitors such as AG1478 and trastuzumab. Oncogenic mutations of p110 have been found at a significant frequency in colon, breast, brain, liver, ovarian, gastric, lung, and head and neck solid tumors. PTEN abnormalities are found in glioblastoma, melanoma, prostate, endometrial, ovarian, breast, lung, head and neck, hepatocellular, and thyroid cancers [72].

Novel patents 2010 – 2011 included compounds which are useful as PI3K protein kinase modulators and in particular as PI3K inhibitors (Table **4**) [73-95].

Table 4. Novel Compounds Patents that Acts as PI3K Modulators and Inhibitor

Patent Number	Type of Modulator/Inhibitor	Inventors
MX 2010012583(A)	Purine compounds and stereoisomers, geometric isomers, tautomers, solvates, metabolites and pharmaceutically acceptable salts thereof, are useful for inhibiting lipid kinases including p110 alpha and other isoforms of PI3K.	Sutherlin *et al.*, 2011
US 2011118257(A1)	PI3K modulators.	Muthuppalaniappan *et al.*, 2011
EP 2316831(A1)	PI3K inhibitors.	Nuss *et al.*, 2011
US 2011098270(A1)	Thiazolopyrimidine compounds selectively inhibit the p110 delta subtype of PI3K.	Hancox *et al.*, 2011
WO 2011054620(A1)	Combined PI3K and MEK inhibitors.	Belvin *et al.*, 2011
US 2011105457(A1)	Heterocyclic inhibitor of PI3K.	Taniyama *et al.*, 2011
EP 2311842(A2)	Compounds inhibitors of P13K/mTOR.	Dong *et al.*, 2011
US 2011083984(A1)	Novel 2,3-dihydroimidazo[1,2-c]quinazoline compounds PI3K inhibitors.	Hentemann *et al.*, 2011
WO 2011041399(A2) Liang, 2011 (Liang, Congxin) (Hentemann Martin, ; Wood Jill, ; Scott William, ; Michels Martin, ; Campbell Ann-Marie, ; Bullion Ann-Marie, ; Rowley Bruce R, ; Redman Aniko) (Taniyama Daisuke, ; Kano Kazuya, ; Okamoto Kazuya, ; Fujioka Masahiko, ; Mitsu	Novel PI3K, especially PI3K delta isoform, selective inhibitors.	
US 2011081316(A1)	Pyrazole inhibitors of PI3K, particularly of PI3K .	Messersmith *et al.*, 2011
US 2011076292(A1)	Benzoxazepin compounds inhibitors of p110 and other isoforms of PI3K.	Blaquiere *et al.*, 2011
US 2011045102(A1)	Cancer chemotherapy compositions comprising PI3K pathway modulators and Triptolide.	Skinner, 2011
US 2011038792(A1)	A method for treating a human cancer based in an inhibitor of a PI3K/Akt pathway protein or its expression is administered to the patient.	Xing, 2011
US 2011112085(A1)	Thiazolopyrimidine PI3K inhibitor compounds and methods of use.	Castanedo *et al.*, 2011
US 2011097349(A1)	Novel PI3K selective inhibitors and method for use.	Bayliss *et al.*, 2011
CA 2736361(A1) (Garcia-Echeverria, Carlos, ; Maira, Sauveur-Michel) (Bayliss Tracy, ; Chuckowree Irina, ; Folkes Adrian, ; Oxenford Sally, ; Wan Nan Chi, ; Castanedo Georgette, ; Goldsmith Richard, ; Gunzner Janet, ; Heffron Tim, ; Mathieu Simon, ; Olivero Alan, ; Staben Steven, ; Sutherlin Daniel P, ; Zhu Bing-Yan) (Castanedo Georgette M, ; Gunzner Janet L, ; Malesky Kimberly, ; Mathieu Simon, ; Olivero Alan G, ; Sutherlin Daniel P, ; Wang Shumei, ; Zhu Bing-Yan, ; Chuckowree Irina, ; Folkes Adrian, ; Oxenford Sally, ; Wan Nan-Chi)	Compounds inhibitors of P13K/mTOR.	Garcia-Echeverria and Maira Sauveur, 2010

(Table 4) contd….

Patent Number	Type of Modulator/Inhibitor	Inventors
MX 2010010659(A) (Bing-Yan Zhu, ; Alan G. Olivero, ; Richard Goldsmith, ; Tim Heffron, ; Daniel P. Sutherlin, ; Tracy Bayliss, ; Aleksandr Kolesnikov, ; Paul Goldsmith, ; Steven Do, ; Steven Staben, ; Michael Siu, ; Adrian Folkes, ; Neil Pegg)	Benzopyran and benzoxepin compounds that inhibit lipid kinases including p110 and other isoforms of PI3K.	Zhu Bing *et al.*, 2010
MX 2010008686(A)	Pyrrolopyrimidin derivative for use as PI3K inhibitor.	Murata *et al.*, 2010
WO 2011008487(A1)	Pyrimidinones that modulate the activity of PI3K.	Li Yun *et al.*, 2011
WO 2010135504(A1)	Thiazolopyrimidinone derivatives for inhibition of PI3K.	Lin *et al.*, 2010
WO 2010059788(A1)	Pyrazolopyridine compounds that inhibits PI3K.	Dotson *et al.*, 2010
US 2010152211(A1)	Methods of inhibiting PI3K- isoform.	Sadhu *et al.*, 2010
WO 2010027002(A1)	Ring-fused morpholine derivative having PI3K-inhibiting activity.	Fujioka *et al.*, 2010

Mutations in genes implicated on PI3K pathway were identified in different tumors. Some patents related identification of targets suitable for development of specific therapies and to stratification of patients based on status of these targets (**US 2011059434 A1**). The method is provided for screening test substances for use as anti-cancer agents. A test substance is contacted with an activated protein kinase selected from the group consisting of: PDK1, AKT2, and PAK4. The activity of the activated protein kinase is tested. An additional aspect of the invention is a method of categorizing cancers. The sequence of one or more protein kinase family members is determined in a sample of a cancer tissue [96].

WO 2011009908 (A2) provides a molecular signature for predicting clinical outcome of a patient affected with breast carcinoma, which method comprises determining the expression of proteins including PI3K, Moesin, pAKT, and/or pMAP-K in a biological sample of said patient [97].

5. CURRENT & FUTURE DEVELOPMENTS

The recent publication of the sequence of the human genome will accelerate the discovery of new genetic susceptibility factors for human disease, leading to the development of novel diagnostics and therapeutics. The exhaustive analysis of the human genome sequence will be the focus of the biomedical research community for many years to come.

In some cellular systems, the information suggests that SHP-1 and SHP-2 can closely regulate the activity of NF- B through alter Akt or PI3K cellular pathways.

PI3K contains two domains: p110 catalytic subunit and p85 regulatory subunit, the second inactivate the first. The inhibitory activity is relieved by occupancy of the NH-terminal SH2 domain of p85 by Src family kinases. Subsequently, PI3K phosphorylate Akt and indirectly active NF- B. This process is reversed by SHP-1 and its association with the regulatory p85 subunit of PI3K that serves to inhibit PI3K activity and lead to the phosphorylation of Akt.

Our progressive grasp upon the impact of SHP-1 and SHP-2 over critical pathways of cell physiology and survival will continue to enhance our ability to design and development targeted therapeutic strategies for cancer treatment [98]. Current patents related to signaling pathway that includes somatostatin receptors, phosphotyrosine phosphatases, tyrosine kinases, AKT/PKB and PI3K are focusing in diagnosis, prognosis and treatment. Many recent patented techniques include inhibition, antagonism or alternative therapeutic methods. Furthermore, is necessary to deepen understanding of the molecular mechanisms involved in cancer to develop other alternatives therapies focusing not only on new inhibitors.

ACKNOWLEDGEMENTS

The authors thank Humera Sharif for provides information of patents. Maria M. Tiscornia has a fellowship for doctoral studies of CONICET, Argentina.

DISCLOSURE

The present article is updated version of previous article of Tiscornia *et al.* (2010) [99].

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