

TLR4 AND PI3K/AKT PATHWAY ACTIVATION: NEW APPROACH FOR CANCER DETECTION

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ABSTRACT

Cancer is not just one disease, but a large group of almost 100 diseases. Its two main characteristics are uncontrolled growth of the cells in the human body and the ability of these cells to move about from the original site and extend to distant sites. If the spread is not controlled, cancer can result in death¹. What difficult is its detection in our body and if it can be detected earlier there is possible treatment with the help of complete removal of tumor. On the other side toll like receptors (TLRs) are family of receptor responsible for detection of pathogen associated molecular patterns (PAMPs) and result in induction of immune response. In various studies over-expression of Akt (serine/threonine protein kinase) phosphorylation has been found in various kinds of cancerous cells when encounter of lipopolysaccharide (LPS) with TLR4 happened. We can identify these changes and move further toward the possible detection. Over-expression of Akt phosphorylation may have possible role for cancer detection in future.

KEY WORDS: TLR4 and PI3K/AKT pathway, Cancer.

1. INTRODUCTION

Cancer is a term for diseases in which abnormal cells divide without control and can invade nearby tissues. Cancer cells can also spread to other parts of the body through the blood and lymph systems. There are several main types of cancer. Carcinoma is a cancer that begins in the skin or in tissues that line or cover internal organs. Sarcoma is a cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue. Leukemia is a cancer that starts in blood-forming tissue such as the bone marrow, and causes large numbers of abnormal blood cells to be produced and enter the blood. Lymphoma and multiple myeloma

are cancers that begin in the cells of the immune system. Central nervous system cancers are cancers that begin in the tissues of the brain and spinal cord also called malignancy². Many factors are responsible for cancer and classified under heading of physical, chemical, environmental and genetic factors. These factors ultimately cause defect in signaling system in our body which in turn to cancer in many times. In every signaling pathway various receptors play a central role. There are number of receptors present in our body responsible for proper regulation of signaling pathway so they can work properly. One of the important receptor families are “toll like receptor family” these are group of

receptor responsible for detection of pathogen associated molecular pattern through conserved motifs. Detection of PAMPs helps to induce immune cell through liberation of inflammatory response. TLRs are very important factor and imperfection in this receptor family may cause immunological problem. An important factor responsible for proper regulation of signaling pathway is PI3K/Akt (Phosphatidylinositol 3-kinases/serine/threonine kinase) signaling pathway which helps in cell growth, proliferation, apoptosis and in proper regulation of various cell functions (figure1)³. In many studies over expression of Akt phosphorylation has been shown which indicate possible role of inflammation in elicitation of various types of cancer. Inflammation response may be helpful to know its role in cancer development and development of marker for cancer.

2. PI3K/AKT PATHWAY

Phosphoinositide 3-kinase (PI3K) plays a crucial role in effecting alterations in a broad range of cellular functions in response to extracellular signals. A key downstream effector of PI3K is the serine/threonine kinase (Akt) which in response to PI3K activation, phosphorylates and regulates the activity of a number of targets including kinases, transcription factors and other regulatory molecules. A causal link between activation of PI3K and the process of cellular transformation was first appreciated in the mid 1980's when the oncogenic activity of Middle T antigen of Polyoma virus was linked to its ability to induce PI3K activity⁴.

PI3K/Akt pathway consists of many activator, inhibitor, effectors, and second messenger, because of its many loops and branches the pathway is very complex and

far from understanding. Most important things are activation of PI3K kinase, formation of second messenger, and downstream effect of Akt. Firstly activation of PI3K starts with activation of PI3K, this activation can be accomplished by three different pathways among which two pathways begin with the receptors belonging to the family of receptor tyrosine kinase (RTK) and extracellular growth factor, binding of the factor leads to the dimerization of the receptors monomers. Depending on the receptor different proteins bind to phosphorylated domain. The insulin receptor substrate-1 (IRS-1) binds to the activated insulin like growth factor (IGF) receptor and for simplification this receptor is depicted as dimer. Receptor bound IRS-1 serves as binding and activation site for PI3K, in addition PI3K binds directly to phosphorylated RTK, a completely different mechanism of PI3K activation begin with the small membrane bound GTPase Ras, by binding with GTPase PI3K is activated. At the second level of pathway the second messenger phosphatidylinositol 3-4-5triphosphate (PIP3) is formed, this activation leads to the activation of the serine/threonine kinase (Akt). The active PI3K migrate to the inner side of the cell membrane and bind to PIP2, which is regular component of membrane and anchored by fatty acid with lipid layer of membrane, PI3K is able to phosphorylate PIP2 to PIP3. PIP3 can activate Akt also protein kinase B and its name is over its homologous protein of retrovirus Akt-A⁵.

Downstream effect of Akt: Akt serine/threonine kinase activated by PIP3 is proto oncoprotein with many substrates and effect, best known effect is inhibition of apoptosis or program cell death. Akt binds with BCL2-associated X protein (BAX) and hindrance its ability to

form holes in the outer mitochondrial membrane. In the absence of Akt this holes leads to apoptosis. Another effect is translation of protein synthesis by multistep cascade of events. It begins with the activation of protein Ras homolog enriched in brain (Rheb) which activate mammalian target of rapamycin (mTOR), mTOR itself interact and activate translation factor S6K by binding with large subunit of ribosome and activate translation of mRNA and protein. In addition Akt may lower the concentration of protein FOXO by phosphorylation of FOXO. Phosphorylated FOXO is a substrate of the enzyme ubiquitin ligase which transfer ubiquitin peptide on to the protein, this peptide are symbolized by small dots, subsequently ubiquitinated FOXO is destroyed by proteasome, in this way Akt prevent tumor suppression protein from inhibiting cell proliferation.

3. TLR4 SIGNALING PATHWAY

TLRs recognize a variety of pathogen-associated molecular patterns (PAMPs) through evolutionary-conserved motifs. Binding of PAMPs on TLRs classically leads to activation of inflammation-associated genes. Whereas TLRs can recognize and elicit an appropriate inflammatory response when the PAMPs are recognized by immune cells, inappropriate activation or regulation may lead to chronic inflammatory conditions and diseases⁷. TLRs are known to elicit appropriate immune activation; TLRs are expressed in a large number of immune cells like B-lymphocytes, monocytes, plasmacytoid dendritic cells and at low levels in human respiratory cells as well as epithelial cells. They activate immune responses by sensing microbial structures such as bacterial LPS, viral RNA, and endogenous “danger” molecules released

by damaged host cells. When TLRs comes in contact with these molecule cascades of events get activated. TLRs forward message to Myeloid differentiation primary response gene (88) (MyD88), MyD88 is an adapter protein that mediates signal transduction for most TLRs and leads to activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and Mitogen-activated protein kinase (MAPKs) and production of pro-inflammatory cytokines. One of the most extensively studied TLRs, TLR4, recognizes Gram-negative bacterial LPS as its prototype agonist. In response to LPS, two pairs of intracellular adapter proteins—MyD88 and Toll-IL-1 resistance (TIR) domain containing adapter protein [TIRAP; also known as MyD88 adapter-like (MAL)] and TIR domain-containing adapter-inducing IFN- (TRIF) and a TRIF-related adapter molecule (TRAM)—interact with TLR4 to activate two main signaling pathways, i.e., “MyD88-dependent” and “MyD88-independent.” Both pathways require receptor dimerization, adapter recruitment, and the activation of specific kinase and transcription factors and result in inflammatory gene expression⁶. Signaling pathway of TLR4 start with interaction between TLR4 and Lipopolysaccharide (LPS) which is present on cell wall of gram negative bacteria and through cascade of events it activate inflammatory response through activation of cytokines⁸. High TLR4 and TLR9 mRNA signal intensity was found in the majority of lung cancer specimens. In contrast, tumor-free lung tissue showed lower signal intensity. Consistently, the low amount of TLR4 and TLR9 protein expression was found in tumor-free lung tissue, while they were strongly expressed in lung cancer tissue. There was no

relationship between the expressions of TLR4 or TLR9 and patients' age, gender, smoking, histological type of tumor, lymph node metastasis, and tumor node metastases (TNM) stage. Both mRNA and protein levels of TLR4 and TLR9 were strongly expressed in lung cancer tissue. In addition, some experiment show positive correlation between the expression level of TLR4 and malignancy of lung cancer. These results suggested that TLR4 and TLR9 may be involved in the development of lung cancer which may have the potentials for the treatment of this malignant tumor⁹. However, they are also implicated in some other types of cancer such as ovary¹⁰, stomach¹¹ and the colon¹².

4. CANCER AND AKT PHOSPHORYLATION

The phosphatidylinositol-3'-kinase/Akt (PI3K/Akt) pathway is involved in the proliferation and progression of a number of types of human cancer. Colorectal cancer is the third most common cancer diagnosed among men and women and the second leading cause of cancer death in the United States¹³. Metastatic or recurrent disease is the most common cause of death in this patients¹⁴. Despite extensive research into the biology of cancer progression, the molecular mechanisms involved in colorectal cancer metastasis are not well characterized. Thus, a thorough understanding of the genetic and epigenetic mechanisms that program metastasis establishment and secondary tumor formation is important for the development and optimal use of novel anticancer therapies. Typically, this pathway is activated when growth factors bind receptor tyrosine kinases (RTKs), leading to activation of PI3K. This active

kinase complex then phosphorylates phosphoinositides, ultimately, leading to Akt phosphorylation and activation. Activation of Akt kinase then leads to modulation of cell growth and metabolism. Some findings suggest that increased TLR4 expression in CRC is consistent with other reports suggesting a correlation between TLR4 and CRC progression. A recent demonstrated that in a mouse model of liver metastases, TLR4 knockdown decreased liver metastasis and tumor burden, particularly in animals known to be more sensitive to LPS¹⁵. It has been reported that the PI3K-Akt axis plays a central role in TLR2-induced activation of neutrophils¹⁶. Activation of Akt in neutrophils stimulated with the TLR2 ligands peptidoglycan (PGN), a product of Gram-positive bacteria, and the lipopeptide tri-palmitoyl-S-glycerol-Cys-Ser-(Lys) (PAM) was of greater magnitude than that stimulated by the TLR4 agonist LPS. The release of the pro-inflammatory mediators TNF- α and macrophage inflammatory protein-2 (MIP-2) by TLR2 agonist-activated-neutrophils was inhibited by blockade of PI3K. Interestingly, PI3K blockade did not inhibit nuclear translocation of NF- κ B in TLR2 ligand-activated neutrophils, but did prevent Ser (536) phosphorylation of its p65 subunit, an event required for maximal transcriptional activity of NF- κ B. Inhibition of PI3K also prevented activation of p38 MAPK and extracellular receptor-activated kinase 1/2 in TLR2-stimulated neutrophils. Therefore, TLR2 on neutrophils participates in Gram-positive bacteria induced acute inflammation, which is mainly mediated by PI3K pathway¹⁷. Some studies have shown that PI3K acts through its downstream effector protein Akt to contribute to colorectal cells (CRC) proliferation and metastasis. The

PI3K/Akt pathway is aberrantly regulated in CRC in a stage-dependent manner and those Akt components, in combination with PTEN suppression, were important in establishing CRC liver metastases. PI3K inhibition exhibits a potent antitumor effect in certain cancer cells including colon cancers; these effects appear to be due to inhibition of Akt/PKB phosphorylation. Increased PI3K activity plays an important role in the increased proliferation and survival of various cancers, including colon cancers. Conversely, the inhibition of PI3K contributes to differentiation of certain cancer cells. As studies have shown that the inhibition of PI3K enhances NaBT-mediated differentiation in human colon cancer cells. In some studies researcher demonstrate that inhibition of the PI3K/Akt pathway enhances NaBT-induced apoptosis in KM20 and HCT116 human colon cancer cell lines {(derived from a Dukes' D colon cancer) was obtained from Dr. Isaiah Fidler (M. D. Anderson Cancer Center, Houston, TX¹⁸)} in-vitro. Either wortmannin or LY294002 in combination with NaBT increased activation of caspase-9 and caspase-3 and the subsequent cleavage of PARP in KM20 cells. Inhibition of PI3K also increased the sensitivity of KM20 cells to either gemcitabine or 5 -FU. Moreover, wortmannin alone inhibited KM20 tumor growth in vivo; the combination of wortmannin and NaBT completely suppressed growth of KM20 tumor xenografts. These findings suggest that agents targeting the PI3K pathway may represent novel adjuvant therapy for the treatment of certain colon cancers¹⁹.

5. PRESENT SCENARIO

Evolving studies with several different targeted therapeutic agents are

demonstrating that patients with genomic alterations of the target, including amplification, translocation and mutation, are more likely to respond to the therapy. Recent studies indicate that numerous components of the phosphatidylinositol-3-kinase (PI3K/Akt) pathway are targeted by amplification, mutation and translocation more frequently than any other pathway in cancer patients, with resultant activation of the pathway. In contrast to p53 and other tumor-suppressor pathways, the PI3K pathway is activated in cancer, making this an optimal target for therapy as it is easier to inhibit activation events than to replace lost tumor-suppressor function. More than 20 companies and academic centers have declared active programs in this area (TABLE I). As PI3K pathway inhibitors are integrated into clinical practice, it will be crucial to develop methods to identify those patients with tumors 'driven' by molecular abnormalities that can be exploited by these inhibitors. As it is not presently clear whether patients with aberrations of particular isoforms or molecules in the pathway will require treatment with drugs targeting particular points in the pathway, it will be important to co-develop molecular markers and targeted therapeutics. This approach will maximize the efficacy and cost effectiveness of these new therapies and minimize needless patient exposure. Further, ability to pre-select patients, who are likely to respond, and to identify patients not responding at an early point during treatment and triage them to alternative therapies, will greatly increase the likelihood of demonstrating efficacy and decrease the size, cost and duration of clinical trials while concurrently protecting patent life, an approach favored by drug companies and recently the FDA. Once a targeted therapeutic is approved

for use in a selected population of patients, post-marketing studies can be used to identify the spectrum of diseases and combination therapies most effective²⁰. At present many studies are in progress related with cancer and Akt phosphorylation and one of the important factors is that cancer is not only responsible for Akt phosphorylation as but it is also related with immune system and many other agent may cause its phosphorylation.

In the traditional model, estrogen modulates the expression of downstream genes by binding to the ER and inducing subsequent nuclear translocation of the receptor dimers. It has been commonly believed that estrogen affects cell signaling mainly through nuclear events. Recently, evidence of the cytoplasmic function of estrogen has emerged. The most striking example is the direct association between the ER and PI3K in cells stimulated by estrogen²¹. Activation of PI3K by this mechanism resulted in activation of Akt and downstream antiapoptotic signaling. On the other hand, exposure to estrogen activates the PI3K pathway and attenuates glutamate-induced toxicity in neuronal cells²². Although the precise mechanism for estrogen-mediated protection in neuronal cells is not clear, the activation of PI3K signaling by estrogen appeared to be ER-independent because estrogen antagonist ICI 182,780 failed to block the protective effect of estrogen. Given the pleiotropic nature of the estradiol hormone, it is possible that estrogen may convey its multiple functions through both ER-dependent and ER-independent pathways²³. Some researchers show the effect Akt phosphorylation on cancer as pAkt significantly predicts disease-free benefits from the sequential addition of paclitaxel to AC chemotherapy in patients

with node-positive breast cancer. Patients with pAkt-negative breast tumors do not appear to benefit from the addition of paclitaxel²⁴. According to one research Perifosine is a membrane targeted alkylphospholipid developed to inhibit the PI3K/Akt pathway and has been suggested as a favorable candidate for combined use with radiotherapy. They investigated the effect of the combined treatment of perifosine and radiation (CTPR) on prostate cancer cells in vitro and find out the result that in vitro, CTPR had greater inhibitory effects on prostate cancer cell viability and clonogenic survival than perifosine or radiation treatment alone. A marked increase in prostate cancer cell apoptosis was noted in CTPR. Phosphorylation of AKT-T308 AKT and S473 was decreased when using perifosine treatment or CTPR. Cleaved caspase 3 was significantly increased in the CTPR group. In vivo, CTPR had greater inhibitory effects on the growth of xenografts when compared with perifosine or radiation treatment groups alone. Perifosine enhances prostate cancer radiosensitivity in vitro and in vivo. These data provide strong support for further development of this combination therapy in clinical studies²⁵. The EGFR-activated phosphatidylinositol 3-kinase/Akt (PI3K/Akt) pathway has been proposed to protect cells from radiation-induced apoptosis by multiple mechanisms²⁶. Deregulation of the PI3K/Akt pathway is often associated with tumorigenesis^{27, 28} and poor prognosis in cancer patients²⁹⁻³¹. In addition, the PI3K/Akt pathway has been implicated extensively as a contributor to radioresistance³². These insights present the PI3K/Akt pathway as an attractive target for anticancer therapy, and more importantly, for combined treatment therapy.

6. CONCLUSION

The study of any pathway is itself a difficult work, there are number of factors that directly or indirectly regulate that pathway and detection of single component in any pathway is become difficult. On the other hand we can't say that any variation in functioning of pathway is only because of any single factor. The factors are interconnected. Beside of many difficulties the pathway analysis is become very popular techniques these days and with the help of biological marker we can identify the key factor. As already discussed, Akt phosphorylation play many important roles in various cell function and it's over expression has been seen in many type of cancers. We can work upon the marker design for cancer which will be related with Akt phosphorylation. PI3K/Akt pathway is a major point to develop biomarkers for cancer. According to a study a research team lead by Merck scientists has taken what could represent a first step toward using phosphoproteins as biomarkers for cancer drug development. Examining prostate cancer cells treated with three investigational drugs targeting the PI3K, signaling pathway, the researchers identified 375 nonredundant serine-threonine phosphopeptides within the pathway, of which 71 were regulated by at least one drug and 11 were regulated by all three drugs. The results, published in a study suggested that biomarker panels comprised some of the drug responsive phosphoproteins that could be used as tools for studying and evaluating the effectiveness of PI3K pathway inhibitors³³.

Many researchers have been working on development of drug on the basis of relationship of Akt phosphorylation and

cancer. As cancer is responsible for number of deaths every year in India as well as other parts of the world, we have to develop a drug which can work effectively at least on cancer detection, so that on the basis of its early detection we can go for its treatment.

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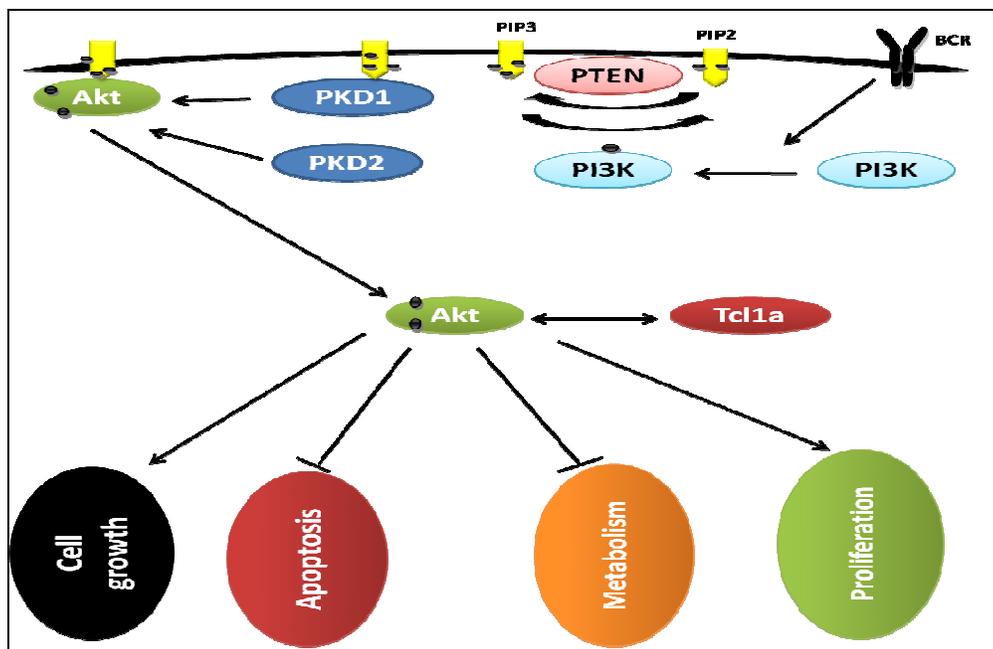


Figure 1 Role of PI3K/Akt pathway in various cell functions.
 (● -Phosphate)

Source- <http://knol.google.com/k/protein-kinase-b#>

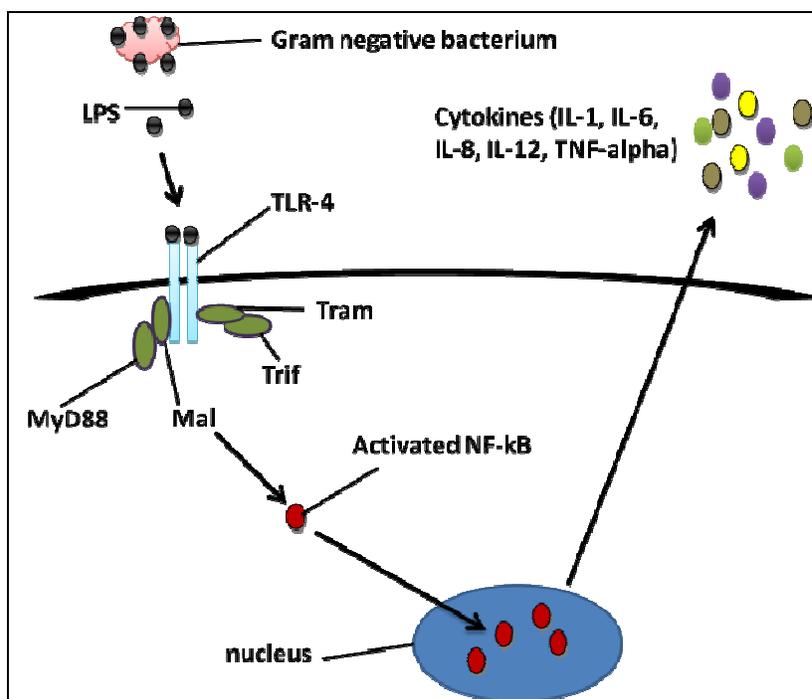


Figure II. Signaling pathway of TLR4

Source-

<http://student.ccbcmd.edu/courses/bio141/lecguid/unit4/innate/images/LPSTLR4signal.jpg>⁸

Table 1- Some biomarker with specific target and name of the manufacturer company²⁰

Effect on pathway	Target	Examples	Company/centre	Status
Direct	PI3K P110 δ P110 α Pan-inhibitor	Ly294002 Wortmannin analogues PX-866 SF1124 PEG Wortmannin KN309	Lilly PROLX Lilly Semafore Echelon Wyeth Baxter ICOS PIramed PIramed Cerylid Cerylid/Kinacia	Poor pharmacology Preclinical Preclinical leads Preclinical leads Preclinical Preclinical Preclinical Preclinica
	PDK1		Berlex Lilly ICOS Vertex	All are preclinical
	ILK		QLT	Preclinical
	AKT kinase domain PH domain	 PX316 Miltefosine Perifosine	QLT Abbott Novartis Lilly Vertex Roche Celgene Novartis Kinacia/Cerylid BioImage PROLX Zentaris Keryx	Preclinical Screening Preclinical Approved in Europe for breast cancer In clinical trial for leishmaniasis Phase II Preclinical

			NIH Schering Merck Celgene	
	mTOR	Rapamycin CCI779 Rad 001 AP23573 AP23841 AP23573	Wyeth Wyeth/NCI/CTEP Novartis Ariad Ariad Ariad	Approved Phase II Phase II Phase II Preclinical
	P70S6 kinase		Lilly	
	Forkhead translocation	Calmodulin inhibitors	Harvard Bioimage	Clinical trials Preclinical
Indirect	Growth factor receptors	EGFR HER2 Insulin Integrins	Multiple	Preclinical to approved
	Intracellular kinases	Src Abl	Multiple	