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Editorial

Therapeutic targets for cancer: Current concepts with PI 3-K, Akt, & mTOR

Phosphoinositide 3-kinase (PI 3-K), protein kinase B (Akt), and the mammalian target of rapamycin (mTOR) significantly impact multiple biological functions that influence cellular development, survival, and demise[1]. Given the ability of these pathways to foster robust cellular proliferation, PI 3-K, Akt, and mTOR have become compelling targets for the treatment of tumourigenesis that can occur throughout the body. Receptor tyrosine kinase (RTK) and the G protein-coupled receptor (CPCR) are required for the activation of PI 3-K. Once active, PI 3-K phosphorylates membrane lipids and mediates the translocation of Akt from the cytosol to the plasma membrane by promoting the binding of Akt to PI-3,4-P_2 and PI-3,4,5-P_3 through the plectrin homology (PH) domain. Akt is subsequently activated through the phosphorylation of its residues serine^{473} and threonine^{308} by phosphoinositide dependent kinase (PDK) PDK1 and PDK2.

mTOR is a serine/threonine protein kinase (289 kDa protein) that is dependent upon the activation of the PI 3-K and Akt cascade. mTOR signaling also relies upon the protein complexes mTOR Complex 1 (mTORC1) or mTOR Complex 2 (mTORC2), each of which contains mTOR and a number of regulatory proteins. Phosphorylation of mTOR can occur at several locations. The C-terminal domain with sequence homology to the catalytic domain of the PI 3-K family contains several phosphorylation sites that regulate mTOR activity, including serine^{2448} that is a target of Akt[2]. Another regulatory component of mTORC1 linked to Akt involves the proline rich Akt substrate 40 kDa (PRAS40) that contains a consensus sequence (RXRXXS/T) for Akt. Akt phosphorylates threonine^{246} on PRAS40 and results in the dissociation of PRAS40 from mTORC1[3]. This process leads to the binding of phosphorylated PRAS40 to protein 14-3-3 to inhibit PRAS40 and activate the mTOR pathway. Tuberous sclerosis complex (TSC) 1 (hamartin)/TSC2 (tuberin) complex also is a target for the modulation of mTORC1 activity. In the absence of Akt activity, the TSC1/TSC2 complex is a negative regulator of mTORC1. TSC2 functions as a GTPase-activating protein (GAP) that converts a small G protein Ras homologue enriched in brain (Rheb-GTP) to the inactive GDP-bound form (Rheb-GDP). The active form Rheb-GTP can result in mTORC1 activation. Akt phosphorylates TSC2 on multiple sites that leads to the destabilization of TSC2 and disruption of its interaction with TSC1. The phosphorylation of TSC2 on the residues of serine^{939}, serine^{981}, and threonine^{1462} can increase its binding to protein 14-3-3 and lead to cellular sequestration, disruption of the TSC1/TSC2 complex, and subsequent activation of Rheb and the mTOR pathway. In addition, Akt can promote the activation of mTORC1 and the mTOR pathway through I-kappaB kinase - α (IKKα). IKKα regulates mTOR activity by associating with Raptor, (regulatory-associated protein of mTOR), that is Akt dependent.

Apoptosis and autophagy

The PI 3-K, Akt, and mTOR pathways can influence cell survival through both apoptosis and autophagy[4]. Following activation through PI 3-K, Akt can block apoptosis and foster cell growth and survival, regulate cellular metabolism, mitochondrial signaling, and cancerous cell growth[5,6]. Activation of mTOR is usually protective against apoptosis especially during periods of oxidative stress. For example, exposure to hydrogen peroxide impairs mTOR kinase activity and leads to apoptotic cell death. In addition, inflammatory

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cells can succumb to the toxic effects of oxidative stress if deprived of mTOR activation. In contrast, mTOR activation through application of nutrients such as phosphatidic acid can limit oxidative stress and prevent apoptotic cell injury. In regards to autophagy, 33 autophagic related genes (*Atg*) have been identified in yeast. Atg1 and Atg13 are associated with the PI 3-K, Akt, and mTOR cascade. In particular, mTOR modulates autophagy through the regulation of these autophagic genes. mTOR can phosphorylate the mammalian homologue of Atg13 (autophagy related gene 13) and the mammalian Atg1 homologues ULK1 (UNC-51 like kinase 1) and ULK2 to prevent the progression of autophagy. The focal adhesion kinase family interacting protein of 200 kDa (FIP200) has been identified as a ULK binding protein[7,8]. Both FIP200 and Atg13 are critical for the stability and activation of ULK1. Mammalian Atg13 binds to ULK1/2 and FIP200 to activate ULKs and facilitate FIP200 phosphorylation by ULKs. As a result, it is suggested that mTOR activation prevents autophagy in mammalian cells through the phosphorylation of Atg13 and ULKs, thus inhibiting the ULK-Atg13-FIP200 complex. During inhibition of mTOR, dephosphorylation of ULKs and Atg13 ensues, leading to the induction of autophagy.

In light of the proliferative roles PI 3-K and Akt hold for cellular growth, it may come as no surprise that these pathways are considered optimal targets to block tumourigenesis. Inhibition of the PI 3-K and Akt pathways may be desirable to promote apoptosis and control tumour progression. Experimental strategies targeted to block activation of the PI 3-K-Akt pathway can suppress medulloblastoma growth, reduce colorectal cancer growth, increase radiosensitivity in tumours, and benefit patients with gynaecological malignancies[9]. In addition, inhibition of the PI 3-K-Akt pathway can also target tumour growth through the induction of autophagy. In oral squamous carcinoma cell lines, application of the agent erufosine that blocks Akt activity leads to cell death through autophagy[10]. Similar results of Akt inhibition that lead to autophagic cell death have been reported in ovarian cancer with other treatments[11].

Inhibition of mTOR can also slow tumour progression during urothelial carcinoma, neuroendocrine tumours, breast and gynaecological malignancies, and solid tumours[12]. In contrast, activation of mTOR pathways with mTORC1 and mTORC2 may contribute to leukemic cell resistance during chronic myelogenous leukemia and colorectal cancer metastases. Increased activity of mTOR has been linked to cancer syndromes such as neurofibromatosis type 1 (NF1) and tuberous sclerosis (TS). In NF1, investigations suggest that increased activity of mTORC1 with impairment of mTORC2 activity occurs in human arachnoid and Schwann cells[13]. Associated bone pathology in NF1 is the result of hyperactive mTOR pathways. During treatment with rapamycin, an inhibitor of mTOR, aggressive NF1-associated malignancies are blocked in genetically engineered murine models of the disease[14]. Increased activation of the mTOR pathway also occurs in TS leading to cortical tubers consisting of giant cells, dysmorphic neurons, and astrocytes. The United States Food and Drug Administration (FDA) has approved everolimus (RAD001), an inhibitor of mTOR, for the treatment of subependymal giant cell astrocytoma which can lead to reduction in tumour volume, resolution of hydrocephalus and gait disorder, and cessation of seizures[15,16]. Inhibition of mTOR with rapamycin in TS patients can also lead to the reduction of facial angiofibromas. The FDA has extended approval of rapamycin (sirolimus) and several rapamycin derivative compounds ("rapalogs") for the treatment of renal cancer (everolimus, temsirolimus) and neuroendocrine pancreatic tumours (everolimus). Present clinical investigations with everolimus for the treatment of advanced neuroendocrine tumours, subependymal giant cell astrocytoma, and epilepsy indicate that inhibition of mTOR can significantly improve clinical outcome[15,16].

Broad implications for the development of effective therapeutic strategies

Despite these encouraging observations, there are concerns for the development of effective strategies against cancer targeting the PI3-K, Akt, and mTOR pathways. For example, studies suggest that chronic inhibition of mTOR may lead to impairment of potential regenerative pathways and metabolic pathways. Prolonged inhibition of mTOR can negatively affect stem cell development, neuronal cell survival in the nervous system, and result in glucose intolerance, hyperinsulinaemia, and hyperglycaemia. Further, cognitive impairment and the loss of synaptic plasticity may be consequences of long-term mTOR inhibition. Treatments with agents that block mTOR activity also lead to multiple side effects that include immunosuppression, oral and respiratory infections, stomatitis, hypertriglyceridaemia, hypercholesterolaemia, and leukopenia[16-18]. This has led

to a concerted focus upon the development of mimetics of mTOR signaling that can meet treatment objectives but eliminate the detrimental effects of current mTOR inhibitory agents.

Clinical studies have suggested that mTOR inhibition may be successful for only subpopulations of patient groups, such as for the treatment of subependymal giant cell astrocytoma or epilepsy[15,16]. This may be due to the development of resistance to agents that inhibit mTOR signaling. In other scenarios, some cancers produce increased PI 3-K and Akt activity in addition to enhanced mTOR activation[19], limiting the efficacy of agents that only target mTOR alone. Inhibition of mTOR signaling pathways in cancer can promote activation of PI 3-K, Akt, and Ras-mitogen activated protein kinase (MAPK) signaling that foster further tumourigenesis. As a result, broad targeting of the PI 3-K-Akt-mTOR axis has been proposed[20]. In preclinical and clinical studies, inhibition of the PI 3-K-Akt-mTOR axis or targeting both mTORC1 and mTORC2 increases radiosensitivity against tumour cell growth[21] and may be beneficial for patients with haematological malignancies and colorectal cancer metastases[22]. Other classes of agents are also under consideration that can target different Akt classes with the alkyl-lysophospholipids and small molecule inhibitors of Akt, as well as combined targeting of mTORC1 and mTORC2 with or without PI 3-K inhibition.

Novel pathways that involve wingless signaling and Wnt1 inducible signaling pathway protein (WISP) are also coming under scrutiny, since these pathways appear to exert a fine modulatory control over PI 3-K, Akt, and mTOR. Studies demonstrate that WISP, a member of the CCN family of proteins, can impact cell survival as well as control unchecked cell proliferation that occurs in cancer. The CCN family of proteins is termed by the first three members of the family that include Cysteine-rich protein 61, Connective tissue growth factor, and Nephroblastoma overexpressed gene and consists of six secreted extracellular matrix associated proteins. In the CCN family, WISP1 (CCN4) may have a reparative and regenerative role during cell injury that relies upon the PI 3-K-Akt-mTOR axis[23]. WISP1 can stimulate lung tissue repair, promote cardiomyocyte proliferation, prevent cell death during bone fractures, block oxygen-glucose deprivation injury in primary neuronal cells, limit amyloid toxicity in inflammatory cells, and may be required for pancreatic and β-cell regeneration during diabetes mellitus. Yet, WISP1 may also be necessary to prevent tumour cell proliferation such as during melanoma cell growth. Recent study suggests that the degree of mTOR activation may determine whether pathways such as WISP foster cell survival or lead to apoptotic cell death[24]. In such a scenario, pathways that finely regulate mTOR activity, such as TSC2, appear to be important targets for the treatment of cancer as well as other disorders that may involve neurodegeneration. It is becoming increasing clear that the successful development of new therapeutic strategies that can effectively block the onset or progression of different forms of cancer in the body will require a broad consideration of not only the components of the PI 3-K-Akt-mTOR axis, but also the ability of each of these components to finely modulate biological function that will ultimately translate into clinical outcome.

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