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Review Article

PI3K/Akt/mTOR signaling pathway in cancer stem cells: from basic research to clinical application

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Abstract: Cancer stem cells (CSCs) are a subpopulation of tumor cells that possess unique self-renewal activity and mediate tumor initiation and propagation. The PI3K/Akt/mTOR signaling pathway can be considered as a master regulator for cancer. More and more recent studies have shown the links between PI3K/Akt/mTOR signaling pathway and CSC biology. Herein, we provide a comprehensive review on the role of signaling components upstream and downstream of PI3K/Akt/mTOR signaling in CSC. In addition, we also summarize various classes of small molecule inhibitors of PI3K/Akt/mTOR signaling pathway and their clinical potential in CSC. Overall, the current available data suggest that the PI3K/Akt/mTOR signaling pathway could be a promising target for development of CSC-target drugs.

Keywords: Cancer stem cells, PI3K/Akt/mTOR signaling pathway, self-renew, tumor initiation, rapamycin

Introduction

In the past decades, cancer stem-like cells (CSCs) have been identified in several types of solid tumors, such as those of the lung, breast, colon and liver [1-4]. CSCs are a unique subpopulation not only a renewable source of tumor cells, but also a source of tumor resistance leading to tumor recurrence, metastasis, and progression [5]. Some key signaling pathways, including Wnt/β-catenin, STAT3 and TGF-β, have been implicated in the maintenance of CSCs [6-8]. The phosphatidylinositol-3-kinase (PI3K)/Akt and the mammalian target of rapamycin (mTOR) signaling pathways are crucial to many physiological and pathological conditions, such as cell proliferation, angiogenesis, metabolism, differentiation and survival [9]. Activate mTOR are frequently improperly regulated in most human cancers. For example, PI3K/Akt/mTOR pathway is activated in approximately 70% of ovarian cancers [10]. Tapia et al. [11] also found that the PI3K/Akt/mTOR pathway is activated in tumor tissues from patients with advanced gastric cancer compare with that in nontumor gastric mucosa. Apart from the attention on cancer cell, more and more recent studies have shown the links between PI3K/Akt/mTOR signaling and CSC biology [12, 13]. In the study of Sunayama et al [14], mTOR signaling has been shown to maintain the self-renewal and tumorigenicity of glioblastoma stem-like cells. In sharp contrast, mTOR inhibition by rapamycin has been shown to significantly increase CD133 expression in gastrointestinal cancer cells [15].

The objective of the present work is to review the evidence about the roles of the PI3K/Akt/mTOR pathway in cancer stem cells and to solve the controversy among these reports.

Pathogenesis of cancer and the PI3K/Akt/mTOR pathway

Previous studies showed the importance of mTOR pathway in cancer pathogenesis. PIK3 is overexpressed in ovarian [16] and cervical cancer [17]. Its mutations have been observed in breast cancer, glioblastoma and gastric cancer [18]. Akt1 overexpression has been detected in gastric carcinoma [19], and Akt2 overexpression has been observed in ovarian and pancreatic cancer [20, 21]. Although mutation of Akt

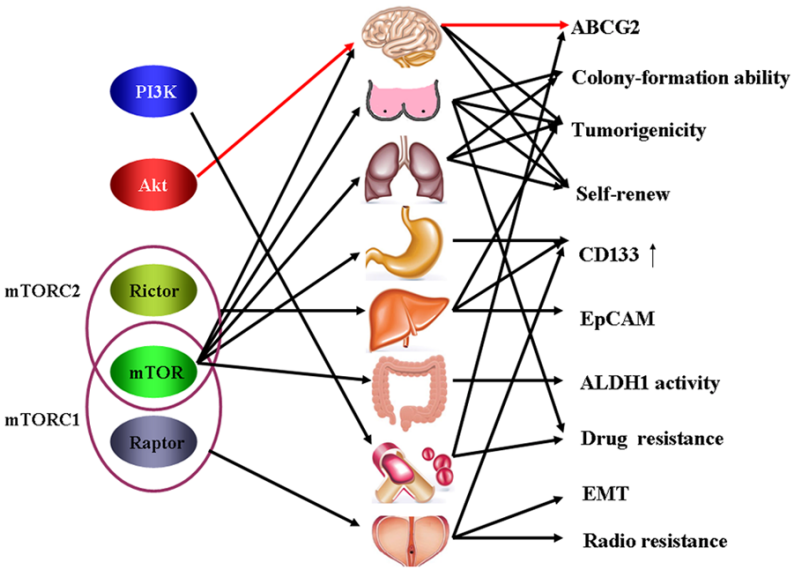

Figure 1. Schematic representation of the PI3K/Akt/mTOR signaling pathway and CSC biology.

itself is rare, Carpten et al. [22] described somatic mutations occurring in Akt1 in a small percentage of human breast, ovarian, and colorectal cancers. mTOR complex 1 (mTORC1) could increase mRNA translation, protein synthesis and cellular proliferation [23]. Activation of a second mTOR complex (mTORC2), involved in regulation of the cytoskeleton, is probably an effect of Akt loop feedback [23]. Balsara et al. [24] found that positive staining for mTOR was exhibited in 74% specimens from the patients with non-small cell lung cancer (NSCLC) by using tissue microarray (TMA). Rictor, a mTORC2 subunit, promoted mTORC2 assembly and activity and endowed glioma cells with increased proliferative and invasion potential [25].

Cancer stem cell and the PI3K/Akt/mTOR pathway

More and more studies showed the role of mTOR pathway in the maintenance of CSCs. Chang et al. [26] found that prostate cancer radioresistance is associated with epithelial-mesenchymal transition (EMT) and enhanced CSC phenotypes via activation of the PI3K/Akt/mTOR signaling pathway. Activation of the mTOR pathway in breast cancer stem-like cells is required for colony-formation ability *in vitro* and tumorigenicity *in vivo* [27]. mTOR suppression could decrease aldehyde dehydrogenase 1 (ALDH1) activity, which is a marker for colorectal cancer stem cells [28, 29]. Inhibition of mTORC2 led to decrease a hepatic CSC marker (epithelial cell adhesion molecule, EpCAM) expression and little or no tumorigenicity in hepatocellular cancer stem cells [30]. Sunayama et al. [14] found that cross-inhibitory regulation between the MEK/ERK and PI3K/mTOR pathways contributed to the maintenance of the self-renewal and the tumorigenic capacity of glioblastoma cancer stem-like cells. Bleau et al. [31] found that Akt, but not its downstream target mTOR, regulates ATP binding cassette transporters (ABCG2) activity in glioma tumor stem-like cells. Corominas-Faja et al. [32] used Yamanaka's stem cell technology in an attempt to create stable CSC research lines, and they found that the transcriptional suppression of mTOR repressors is an intrinsic process occurring in luminal-like breast cancer cells during the acquisition of CSC-like properties. Previous studies have indicated that CD133 is one of the markers for cancer stem cells [33-36]. Inhibition of mTOR signaling up-regulated CD133 expression in gastrointestinal cancer cells [15]. The results of Yang et al. [37] showed that mTOR inhibition increase the $CD133^+$ subpopulations, and trigger the conversion of $CD133^-$ to $CD133^+$ liver tumor cells. These two results indicated that inhibition of mTOR signaling could induce the generation of CSC cells. However, the main reason for the discrepancy is different cellular contexts. CD133 expression mRNA and protein levels were elevated under hypoxic conditions [38].

Dubrovska et al. [5] found that PTEN/PI3K/Akt pathway is critical for prostate cancer stem-like cell maintenance and that targeting PI3K signaling may be beneficial in prostate cancer treatment by eliminating prostate cancer stem-like cells. Activated PI3K upregulated ABCG2 expression and elevated percentage of cancer stem-like cells in both acute myeloid leukemia

(AML) and acute lymphoblastic leukemia (ALL) [39]. However, in the study of Airiau et al. [40], they found that mTOR inhibition showed no effect on chronic myeloid leukemia (CML) stem cells ($CD34^+/CD38^-$), while PI3K inhibition restored the cell line sensitivity to nilotinib, a second generation tyrosine kinase inhibitor (TKI). Abnormal activation of PI3K/Akt/mTOR signaling pathway leads to enhanced expression of chemokine (C-X-C motif) receptor 4 (CXCR4), which in turn promotes CXCR4-mediated STAT3 signaling that might be responsible for maintenance of stemness in NSCLC cells [41]. Chang et al. [42] found that insulin-like growth factor-1 receptor (IGF-1R) and its signaling via PI3K/Akt/mTOR pathway are attractive targets for therapy directed against breast cancer stem cells. Cyclin G_1-induced liver tumor-initiating cells expansion contributes to the recurrence and chemoresistance of hepatoma via Akt/mTOR signaling [43]. Decreased mTOR activity in response to hypoxia-inducible factor 1α (HIF-1α) upregulation inhibits proliferation and promotes survival of prostate cancer stem cells through the PI3K feedback loop [44].

As discussed above, a link between the PI3K/Akt/mTOR pathway and cancer stem cell is clearly evident and the components of this pathway are viable candidates for therapeutic intervention (**Figure 1**).

PI3K/Akt/mTOR is a target for cancer stem cells therapy

The Food and Drug Administration (FDA) approved temsirolimus for the treatment of advanced stage renal cell carcinoma in 2007. Temsirolimus became the first mTOR inhibitor approved for cancer therapy [45]. From then on, three generations of compounds targeting PI3K/mTOR have already been developed. The first-generation of PI3K inhibitors, also being called "pan-inhibitors", were able to bind all class I PI3Ks [46]. The second-generation inhibitors are characterized by greater and isoform-specific selective activity [46]. The third generation inhibitors, "dual PI3K/mTOR inhibitors", not only inhibits all PI3K class I isoforms, but also mTORC1 and mTORC2 [47].

The mTOR antagonist everolimus has effective inhibitory effects on HER2-overexpressing breast cancer stem cells *in vitro* and *in vivo* by reducing the expression of Akt1 and p-Akt [47]. Liu et al. [48] found that everolimus in combination with letrozole inhibit human breast cancer MCF-7 stem cells via PI3K/mTOR pathway.

Mendiburu-Eliçabe et al. [49] found that rapamycin reduced cell proliferation and tumorigenic potential, led to the loss of $CD133^+$ population and increased the level of p-Akt in glioblastoma cells. Wang et al. [50] found that depletion of F-box and WD repeat domain containing 7 (FBXW7) in colon cancer cells induces EMT and cancer stem cell-like characteristics, which can be suppressed by mTOR inhibitor, rapamycin. Rapamycin also has been demonstrated that could target the self-renewal and vascular differentiation potential in patient-derived hemangioma stem cells [51].

Metformin (1,1-dimethylbiguanide hydrochloride), the most widely prescribed drug for treatment of type 2 diabetes, inhibition of CSCs was first showed in 2009 in preclinical breast cancer models [52]. Interestingly, metformin preferentially kills CSCs over NSCCs (non-stem cancer cells) derived from human breast tumors, and it inhibits growth of mammospheres derived from these tumors [53]. These results were subsequently extended to pancreatic cancer cell line, metformin decreased CSC markers, CD44, CD133, ALDH1, and EPCAM and modulation of the mTOR signaling pathway [13]. Metformin eradicates radioresistant cancer stem cells in mouse fibrosarcoma cell (FSaII) and human mammary adenocarcinoma cell (MCF-7) by activating AMP-activated protein kinase (AMPK) and suppressing mTOR [54]. Furthermore, the proliferation of breast cancer stem cells was markedly suppressed by metformin that leading to inactivation of mTOR [55].

Salinomycin is a monocarboxylic polyether antibiotic used to prevent coccidiosis in poultry [56]. Gupta et al. [57] showed that salinomycin selectively kills human breast CSCs in 2009. A series of followed studies showed similar effects of salinomycin in other types of CSCs, such as pancreatic cancer [58], colorectal cancer [59] and lung cancer [60]. Many mechanisms of salinomycin have been identified in CSC cells [58-60]. One of the mechanisms is that salinomycin induces cell death and differentiation in head and neck squamous cell carcinoma stem cells by activation of EMT and Akt [61]. Metformin in combination with salinomy-

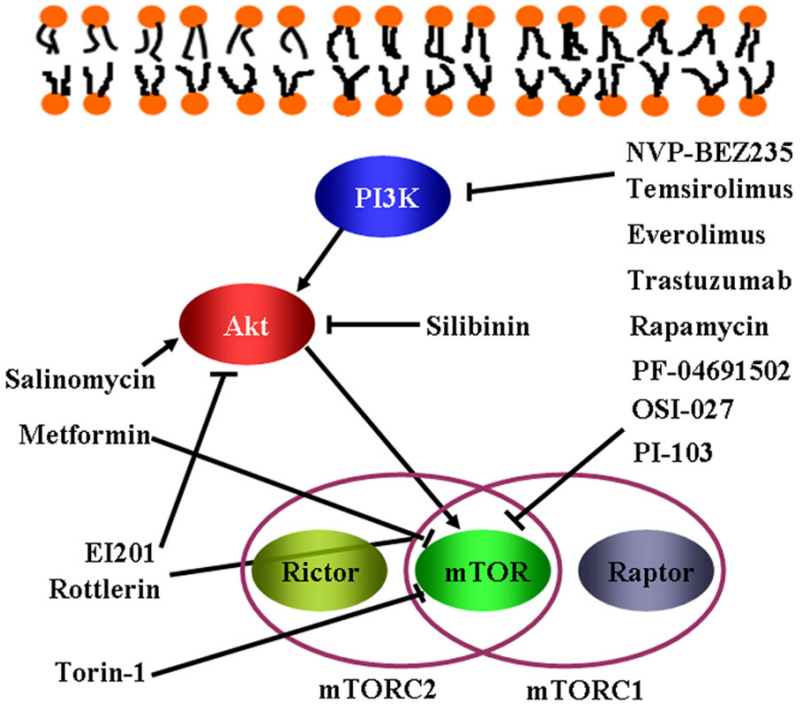

Figure 2. Schematic representation of the action PI3K/Akt/mTOR pathway inhibitors in CSC.

cin could be a promising treatment option for five NSCLC cells and their stem cells (HCC4006, NCI-H1975, NCI-H2122, HCC95 and NCI-H3122) [62].

The radiosensitization efficiency of NVP-BEZ235, a novel dual PI3K/mTOR inhibitor, is achieved in human glioma stem cells by its cumulative antitumor effects, including induction of autophagy, apoptosis, cell cycle arrest, and prevention of DNA repair [63]. Dubrovska et al. [64] also found that NVP-BEZ235 leads to a decrease in the population of $CD133^+/CD44^+$ prostate cancer progenitor cells *in vivo*. Blockage of the PI3K/mTOR pathway inhibited the *in vitro* proliferation of colorectal cancer stem cells and *in vivo* xenograft tumor growth by using a dual PI3K/mTOR inhibitor, PF-04691502 [65]. The apoptosis-inducing mTOR inhibitor, Torin-1, hindered growth, motility, invasion, and survival of colorectal cancer stem cell *in vitro*, and suppressed tumor growth *in vivo* [66]. A novel dual mTORC2/mTORC1 inhibitor, OSI-027, suppresses primitive leukemic precursors from AML patients and is much more effective than rapamycin in eliciting anti-leukemic effects *in vitro* [67]. The anthracycline daunorubicin (DNR) is one of the major antitumor agents widely used in the treatment of AML [68]. PI-103, a dual inhibitor of PI3K and mTOR sensitizes AML stem cells to daunorubicin-induced cytotoxicity [69]. Hong et al. [70] also found that arsenic disulfide (As_2S_2) synergizes with PI-103 eradicated AML stem cells by targeting the PI3K/Akt/mTOR pathway. Silibinin, a flavonoid compound, inhibits colon CSCs self-renewal and sphere formation by suppressing the PP2A/Akt/mTOR pathway [71]. Quinoline imidoselenocarbamate EI201 reduces the CSC population and inhibits tumor growth in an *in vivo* model of prostate cancer by suppressing Akt/mTOR pathway [72]. Rottlerin is a plant derived chemotherapeutic agent and has been used as a protein kinase C-Δ signaling pathway inhibitor [73]. Singh et al. [74] found that rottlerin induces autophagy which leads to apoptotic cell death through inhibition of PI3K/Akt/mTOR pathway in human pancreatic cancer stem cells. Kumar et al. [75, 76] found that rottlerin induces autophagy and apoptosis in both prostate and breast cancer stem cells via PI3K/Akt/mTOR signaling pathway.

Based on the previous studies as described above, better understanding of PI3K/Akt/mTOR signaling should create novel therapeutic opportunities in treating cancer stem cells (**Figure 2**).

Perspective

In summary, the PI3K/AktmTOR pathway is a very complicated intracellular network, we are only at the beginning of understanding the precise role of PI3K/Akt/mTOR signaling in regulating cancer stem cells. Our current understanding of the precise mechanisms through PI3K/Akt/mTOR signaling is still extremely limited. Importantly, it remains to be determined how broadly useful such molecules will be in the clinical setting. We believe that these data will

greatly impact the development of new therapies being designed to eradicate CSC.

Disclosure of conflict of interest

None.

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