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Review Article

Targeting the PI3K-Akt-mTOR signaling pathway involved in vasculogenic mimicry promoted by cancer stem cells

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Abstract: An accumulating body of evidence has led to the development of the cancer stem-cell (CSC) model which proposed that a subset of cells distinct from those that form the tumor mass regulated the tumor growth rate over a long period. Various types of therapy have been developed for cancer treatment. The major conventional therapies are chemotherapy, radiation therapy, and surgical excision. The other emerging therapies include targeted therapy using molecule-based agents. However, the resistance to chemotherapy and radiation therapy frequently occurs. This was most likely due to the dysregulated functioning of the multidrug efflux pumps and nucleotide repair systems resulting from the multiple interactions between the CSCs and the tumor microenvironment. Even though chimeric antigen receptor T-cell and immune checkpoint blockade therapies have succeeded remarkably for treating cancers, evidence suggested that CSCs promoted the development of resistance to these therapies and led to metastasis. The cells with stem cell-like features actively participate in vasculogenic mimicry in different types of cancer. In addition to melanoma, vasculogenic mimicry has been observed in various cancers. One of the major signaling pathways in CSCs is the phosphoinositide 3-kinase (PI3K)/Akt/PTEN pathway. PI3Ks are a family of enzymes that play a critical role in cellular growth, migration, differentiation, and vasculogenic mimicry. The PI3K-Akt pathway also plays a crucial role in epithelial-mesenchymal transition and the establishment of CSC-specific phenotypes through the PTEN/Akt mechanistic target of the rapamycin axis. Thus, targeting the PI3K pathway could be beneficial for cancer treatment through the elimination of CSCs, and such therapy might break niches which maintain the CSC, inhibit the metastasis, and suppress the recurrence of cancer.

Keywords: Cancer stem cells, vasculogenic mimicry, tumor microenvironment, PI3K, epithelial-mesenchymal transition

Introduction

A cancer stem cell (CSC) is identified as a cell capable of initiating human-type acute myeloid leukemia in the NOD/SCID mice, thus demonstrating the capacities of differentiation, proliferation, and self-renewal typically expected of a leukemic stem cell [1, 2]. The CSC hypothesis has been extended to solid cancers, with the first such identification being reported in breast cancers that demonstrated heterogeneity in the population of tumor components and in which a minor subset with the $CD44^{high}$ and $CD24^{low}$ phenotype was capable of generating tumors although they exhibit little morphological differences with the normal cells [3]. Thereafter, CSCs have been found in several cancers including the breast [3-5], colon [6-9], pancreatic [10], and ovarian cancers [11], in addition to malignant brain tumors [12, 13]. CSCs have also been reported in melanoma as a subpopulation of ABCB5-positive human malignant melanoma initiating cells [14]. An accumulating body of evidence led to the development of the CSC model, which proposed that a specific subset of cells distinct from those that form the tumor mass regulated the tumor growth rate over a long period [15]. Therefore, the CSC model was in stark contrast with the stochastic model in that it reflected the hierarchical organization of a healthy cellular system, and several studies have suggested that more

effective targeting of the CSCs may lead to improved patient outcomes [16]. The most widely used cell surface markers for the identification of CSCs include CD44, CD24, CD133, CD326, and CD126 which are highly conserved among the hematopoietic tumors and carcinomas. However, the clinical applicability of CSC markers in solid tumors is relatively limited due to their expression on the surfaces of non-cancerous cells as well.

With regards to the tumor microenvironment (TME), a typical feature of CSCs was the lowering of pH of the TME, induced hypoxicity, reduced nutrition, and increased acidosis which was accelerated by enhanced glucose metabolism and the production of byproducts such as L-lactate [17]. This metabolic rewiring forces tumors to maintain anabolism while controlling the production of reactive oxygen species (ROS) [18]. Other than glucose, fatty acids can also support metabolism in CSCs by serving as alternative fuels for the supply of high-energy phosphoric compounds including ATP and NADH [19]. Therefore, the chemicals that inhibit the fatty acid metabolism pathways in CSCs have been employed in cancer research [20].

For distant metastasis to occur as per the CSC model, the CSCs must first intravasate into the vasculature. To accomplish this process, CSCs must alter their epithelial-cell-like phenotypes such as cell-cell adhesions mediated through certain adhesion molecules including E-cadherin to the mesenchymal cell-like phenotypes of high migration rates and enhanced invasive capabilities [21]. This process called epithelial-mesenchymal transition (EMT) can be one of the possible targets for efficient cancer therapy [22]. The tumor cells accomplish metastasis not only through the direct migration of CSCs but also by their proliferative capability even in a relatively severe microenvironment [23]. A small subset of CSCs called metastasis-initiating or metastatic stem cells disseminate to distant body sites and initiate the development of new tumors [24]. The heterogeneity and plasticity of CSCs pose challenges to the establishment of novel therapeutic interventions against metastasis.

Resistance of cancer stem cells to immunotherapy

Various therapies have been developed for cancer treatment. The major conventional therapies used are chemotherapy, radiation therapy, and surgical excision. Other emerging therapies include targeted therapy using molecule-based agents. However, the resistance to chemotherapy and radiation therapy frequently occurs. This is most likely due to the dysregulated functioning of the multidrug efflux pumps and nucleotide repair systems resulting from the multiple interactions between CSCs and the TME [25]. Such resistance can also be acquired through certain other mechanisms such as an alteration in the redox state and the consequent dysregulation of ROS-signaling pathways. Particularly, the antioxidant enzyme expression plausibly contributed to the modulation of the redox microenvironment, which assisted in preventing the process leading to the development of resistance [25].

Chimeric antigen receptor (CAR) T-cell therapy is a type of cancer immunotherapy that uses engineered cell surface receptors demonstrating a combination of antigen-binding and T-cell activating functions. CAR T-cell therapy is useful to target a specific antigen in cell-based therapies. In CAR T-cell therapy, T cells from the patients are cultivated *ex vivo* and modified to attack tumor cells. This therapy had initially been applied to certain hematogenous tumors but has now been extended to solid tumors and CSCs. The major CSC surface markers including CD44, CD133, and CD326 serve as possible targets for the establishment of novel CAR T-cell therapy. CD133 has been the most well-studied target for CAR T cells of hematogenous tumors such as acute myeloid leukemia. Preclinical experiments have used CAR T cells to treat solid tumors including brain and prostate cancers. Moreover, ongoing clinical trials have targeted CD133 and EGFR, the expressions of which were frequently upregulated in these malignant tumors [17].

Another emerging method of cancer immunotherapy is immune checkpoint blockade (ICB) therapy. This is a promising therapy for cancer treatment, which may assist in the selective elimination of CSC subsets to promote the complete regression of solid tumors [18]. The CSCs exhibit phenotypes including an altered expression of antigens leading to a suppressed recognition by the immune survailance that enables them to circumvent attacks by it. An ICB therapy can be used to target CSCs. A crucial breakthrough in cancer immunotherapy has been the

discovery of immune checkpoint-associated proteins in tumors, namely CTLA-4 (cytotoxic T-lymphocyte-associated protein 4), PD-1 (programmed cell death protein-1) and its ligands PD-L1 (programmed cell death-ligand 1) and PD-L2 [18].

The CAR T-cell and ICB-based therapies have remarkably succeeded for treating cancers; however, evidence has shown that CSCs promoted the development of resistance to these therapies and led to metastasis [26]. BCAT1, an enzyme involved in the reaction of branched-chain amino acids and 2-oxoglutarate, catalyzes the synthesis of glutamic acid and branched-chain 2-oxo acids, which function as key downstream mediators for the production CSCs by IFNγ [26]. The pathway specifically contributes to the production of cellular energy, thus serving as a molecular nexus important for determining the balance between stemness and differentiability in CSCs.

PI3K signaling in cancer stem cells

One of the major pathways of intracellular signal transduction in CSCs is the PI3K-Akt-PTEN axis [27]. This pathway is often dysregulated in many cancers and is supposed to be responsible for determining the fate of the CSCs. It also plays a pivotal role in the cellular proliferation/survival under normal physiological conditions [28]. For instance, the development of prostate cancer is often accompanied by the deficiency of the tumor suppressor PTEN, which negatively regulates the PI3K-Akt activity. This multifunctional PTEN inhibits the PI3K-Akt pathway in the cytosol and stabilizes the genomic DNA in the cell nucleus. Multiple growth factors and nutrients function as regulators for the PI3K-Akt pathway. Upon activation, PI3K associates with other molecules such as Akt which transduces the signaling to the downstream of the cascade [27]. Generally, the signal pathway networks orchestrate the mechanism underlying cell cycle and cell growth. Deficiency in the genes encoding some of the effectors may either enhance or suppress the signaling activities. Alteration in the signal transduction is common in many malignancies; it confers cancer cells with proliferative capabilities and survival advantages. Thus, both the nuclear and cytosol-specific PI3K-Akt activities may be correlated with the pathogenesis of cancer [27]. Furthermore, the PI3K-Akt pathway induced the activation of the gene expression related to multi-drug resistance to tumor cells and was closely relevant to the development of antagonism to therapies, a major hurdle in cancer chemotherapy. Since this signaling pathway may be a promising target for molecule based-targeted therapy of cancer, various pharmacologic inhibitors of the PI3K-Akt pathway have been developed [27]. For example, a PI3K inhibitor NVP-BEZ235 decreased the population of CD133- and CD44-positive human prostate cancer progenitor cells in vivo [29]. This pathway determines the fate of CSCs by playing a dynamic role in maintaining embryonic stem cell pluripotency [30]. It also plays a critical role in EMT and the establishment of CSC-specific phenotypes through the PTEN-Akt mechanistic target of the rapamycin axis. Thus, targeting the PI3K signaling pathway could be beneficial for cancer treatment by eliminating the CSCs (**Figure 1**).

Vasculogenic mimicry and CSCs

Vasculogenic mimicry (VM) was first reported in melanoma [31]. The term refers to the ability of the aggressively proliferating tumor cells to differentiate and acquire an endothelial cell-like morphology for the generation of tumor-derived vascular networks and a microcirculation that was independent of the non-cancerous cells [32]. It was subsequently observed in various cancers including mammary, ovary, pulmonary, kidney, bladder, and prostate cancers; certain sarcomas; and brain tumors including glioma, glioblastoma, and astrocytoma [33]. Gene expression profiling in many cell lines from human melanoma revealed the phenotypes of the aggressively proliferating tumor cells [34]. The transcription patterns of the genes associated with VM shared components with those related to stemness and EMT, which are key attributes conferring tumor plasticity during metastases and resistance to chemotherapy [33]. Cancer cells that are capable of VM often exhibit a plasticity, making the cells to have de-differentiated phenotypes such as stem cells. The VM of malignant tumor cells is accompanied by an undifferentiated biomolecular features harboring embryonic cell-like plasticity in differentiation, which suggests a linkage between cancer stem cells and tumor cells with VM-forming ability. The cells with stem cell-like

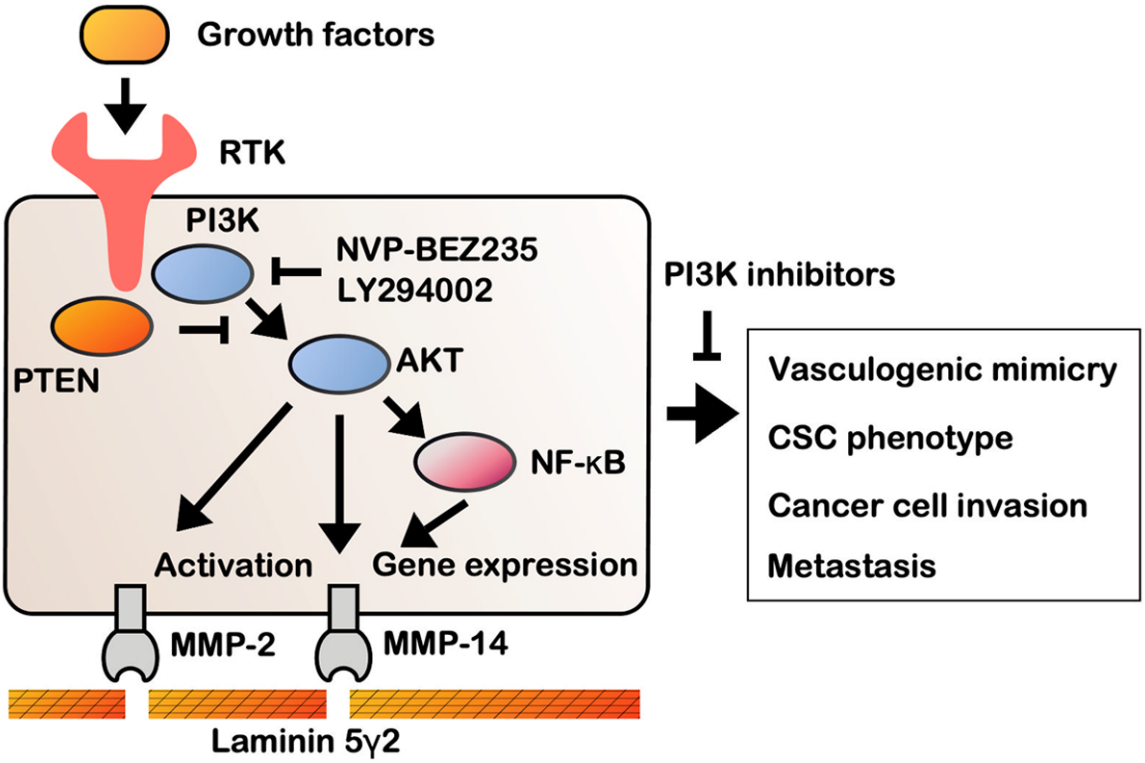

Figure 1. Schematic model of PI3K signaling pathway involved in vasculogenic mimicry (VM) and cancer stem cell (CSC) phenotypes. The PI3K-Akt-PTEN pathway is responsible for determining the fate of the CSCs via receptor tyrosine kinases (RTKs) with growth factors toward cancer cell invasion and metastasis. Therefore, targeting the PI3K signaling pathway could be beneficial for cancer treatment by eliminating the CSCs. Various pharmacologic inhibitors of the PI3K-Akt pathway have been developed: for example, a PI3K inhibitor NVP-BEZ235 decreased the population of CSCs. PI3K has also been identified as a responsible enzyme for activation of MMP-2 and MMP-14, and the laminin isoform processing, which consequently result in VM. A PI3K inhibitor LY294002 inhibited the activity of undifferentiated melanoma cells to undergo VM formation under 3D culture, where LY294002 inhibited the MMP-2 activity as well as the enzyme activity and gene expression of MMP-14 via NF-κB, and consequently suppressed the proteolysis of laminin 5γ2 chain. The data elaborating on the inhibitors effectively used to target CSCs and VM were reported in literatures [29, 36, 37].

features actively participate in VM in different types of cancer. CSC is known to be involved in VM in mammary carcinoma and melanoma [32].

EMT is supposed to be one of the steps of the cascade during metastasis, which starts from initiation, survival of cancer cells under different surroundings, invasion, and finally to form distant metastasis. However, the EMT model is usually applicable to tumors with epithelial phenotype. In contrast, VM has been observed in a broader types of cancers; VM is related not merely to angiogenesis and supply of O_2 for the tumor tissues but to the intravasation and extravasation processes. Comparative microarray analyses of aggressive and nonaggressive breast cancers revealed the remarkable coexpression of several phenotypic markers related to endothelial, mesenchymal, and the stem cells with the suppression in the expression of breast epithelial markers [35]. Such a deeper analysis suggested a phenotypic plasticity of the tumor cells and the imparting of the advantage to tumorigenicity and metastasis. For instance, the cancers harboring such signatures may exhibit de-differentiation out of the epithelium-like phenotype and transit to mesenchymal cell-like characteristics, a process related to EMT. Notably, the upregulation of the expression of endothelial surface proteins by aggressive mammary tumors contributed to VM, describing the formation of non-comparable, vascular-like meshwork by cancer cells, which may help the cancer cells to grow, invade, and form metastasis [33].

During the VM initiation process, the transcription factors belonging to the ZEB family, ZEB1 and ZEB2, bind to regulatory sequences on the E-box of the target gene, and either inhibit or activate its expression. ZEB often recruits the C-terminal binding protein for transcriptional repression. The overexpression of ZEB is also correlated with the development of VM through the promotion of the EMT process. HIF-1α is able to enhance the expression of genes related to the production of molecules, including ZEB2, Snail, Twist, etc. involved in the VM process. These genes are EMT-related transcription factors, which can enhance the synthesis of fibronectin, VE-cadherin, vitronectin, etc., and inhibit that of E-cadherin, thereby promoting EMT and VM.

Vasculogenic mimicry and PI3K signaling

VM is a type of cancer cell's plasticity with many implications. Therefore, the understanding of the molecular mechanism underlying the vascular-mimic network formation of aggressive cancer cells might provide clinicians with novel methods for the therapeutic intervention of tumors. PI3K has been identified as a responsible enzyme for activation of MMP-14 and -2, and the laminin isoform processing, which consequently result in VM, thus involved in the regulation of VM [36] (**Figure 1**).

The most important signaling molecules implicated in VM are VEGF-VEGFR2 and PI3K-Akt-mTOR, which modulate the various important signals that are related to the enhancement of vascular formation, vascular permeability, tube formation, the expression of endothelial markers, and vascular development. The activation of the vasculogenesis-related signal transduction may be associated with the VM promotion. The activities of Ephrin-A2 and VE-cadherin are involved in PI3K-modulated VM, leading to the upregulation in the levels of MMP-14, resulting in the laminin proteolysis [33, 37].

Vasculogenic mimicry and cancer therapy

VM is associated with a poor clinical outcome as it imparts an advantage to tumor survival and perfusion of metastases. Several signaling pathways play crucial roles in tumor-cell-associated VM, including embryonic, stem cell, hypoxia, and vasculogenesis-associated cascades. These signals could be a therapeutic target for metastatic capabilities of tumors [32].

Antiangiogenesis therapy has emerged as possible strategies to treat cancers [38]. The strategy is focused on inhibition of the proliferative and migratory abilities of vascular endothelial cells toward hypoxic sites, where it inhibits the O_2 and nutritional supplementation for cancer cells. Given that certain tumor cells are able to conduct tube formation under 3D cultivation, and the endothelium-related gene expression, VM may be modulated by angiogenesis inhibitors by inhibiting of endothelium-like angiogenesis phenotype. However, treatment with angiogenesis inhibitors did not work on tubular structures formed by aggressive melanoma cells [38]. Interestingly, a study suggested antiangiogenesis strategy might exhibit a tumor response that directs the malignant progression of tumors [39]. These observations imply that antiangiogenesis treatment may initially cause tumor stasis, but may be harmful since the antiangiogenesis inhibitors could allow the tumor to select the cells with the ability to survive under severe hypoxic conditions. Such escaping may be relevant to the plasticity of tumor cells in gaining endothelial cell-specific characteristics of VM. Since VM may play a role as a complementary means of tumor cell diffusion to establish an alternative process for tumor metastases, the observations suggest the possibility of tumors relying on other vascular formation machinery by changing the intrinsic properties under conditions that hamper angiogenesis. To test the feasibility, it is critical to estimate the occurrence of tumor-associated vessel-like channels in future studies. Notably, preclinical experiments showed the induction of a resistant mechanism and tumor development, yet clinical studies so far has not reported an increase in tumor malignancy that follows antiangiogenesis therapies [40]. However, because tumors consist of a variety of cell populations with heterogeneity, it can be assumed that tumors can adapt to develop through selection, providing the insights for the development of more accurate antivascular and antitumor strategies [41].

Conclusions and future perspectives

CSCs may play an important role in VM in a variety of tumors including melanoma and breast

cancer, and CSCs have close association with EMT: as for EMT to occur, the cancer cells need to acquire a stem cell-like properties. Notably, a study on glioma-initiating cells indicated that certain chemical compounds and proteins which modulates EMT also affected the properties of VM [42]. The confirmed association of CSCs with VM may present novel strategies for the optimization of antitumor therapy. VM is the major pathway for O_2 and nutrition to the tumors, and CSCs can differentiate to lining up as tubes, a process resembling VM. However, conventional antiangiogenesis agents including endostatin and angiostatin are not effective against VM in which normal endothelial cells do not exist, while the anti-VEGF monoclonal antibody, Bevacizumab, significantly reduced the CSCs and resulted in the arrest of tumor growth in a glioblastoma experimental model [43]. Hess et al. suggested that a PI3K inhibitor LY294002 impeded the activity of undifferentiated melanoma cells to undergo VM formation in a three-dimensional (3D) culture on type I collagen matrix [36]. They further reported that treatment of 3D-cultured melanoma cells with the agent LY294002 markedly inhibited the MMP-2 activity as well as the enzyme activity and gene expression of MMP-14, and thereby suppressed the proteolysis of laminin 5γ2 chain [37]. They claimed that these results suggest PI3K signaling pathways may positively participate in VM of melanoma cells via the regulation of the activities of the matrix proteinases such as MMP-2 and MMP-14, which resulted in the degradation of laminin 5γ2 chain into promigratory fragments [37] (**Figure 1**).

The VM channel structures can help the cancer cells to expose to the blood flowing through the vasculature facilitating their metastases. VM is frequently observed in the areas between the tumors and surrounding normal tissues and has close relation to a clinical prognosis. Thus, therapies targeting VM may break niches which maintain the CSC, inhibit the metastasis, and suppress the recurrence of cancer. In particular, PI3K-Akt-mTOR signaling pathway involved in vasculogenic mimicry promoted by cancer stem cells may be a promising target for the translational research in cancer treatment. Such therapies might break niches which maintain the CSC, inhibit the metastasis, and suppress the recurrence of cancer, and they will offer promise for the development of new therapeutic intervention strategies to target a wide variety of tumor types.

Disclosure of conflict of interest

None.

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