

Crosstalk Between the PI3K/AKT/mTOR Signaling Pathway And The HIF-1 α /CA9 Axis and its Significance in Leukemogenesis of Acute Myeloid Leukemia

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Abstract

Acute myeloid leukemia (AML) is a refractory heterogeneous hematological disease. Leukemic stem cells (LSCs) in the CD34+CD38- AML cells are causes of therapy resistance and relapse. Progression of LSCs to overt AML is primarily dependent on cell-intrinsic oncogenic alterations. However, bone marrow microenvironment (BMM) also affects leukemogenesis of AML. In fact, activated expression of hypoxia-inducible factor 1 α (HIF-1 α) and carbonic anhydrase IX (CA9) is observed in AML. In addition, constitutive activation of the phosphatidylinositol 3-kinase (PI3K)/AKT/mechanistic target of rapamycin (mTOR) signaling pathway in AML is also detected. This review article aims to summarize the crosstalk between aberrantly activated PI3K/AKT/mTOR signaling and the HIF-1 α /CA9 axis in AML for clarification of AML leukemogenesis. Aberrant activation of PI3K/AKT/mTORC1 signaling that is induced by suppression of phosphatase and tensin homolog (PTEN), activating mutation of *FLT3*, autocrine/paracrine activation of VEGF/VEGFR-2 signaling and autocrine activation of IGF-1/IGF-1R signaling activates translation of HIF-1 α mRNA via activation of 4E-BPs and S6Ks, and transcription of HIF-1 α mRNA via activation of nuclear factor kappa B (NF- κ B). These mechanisms of the crosstalk between the PI3K/AKT/mTORC1 signaling and the HIF-1 α /CA9 axis finally activate CA9. Further research on this crosstalk is required for development of novel therapies against AML LSCs.

Keywords: AML, PI3K, AKT, mTOR, HIF, CA9.

ABBREVIATIONS

4E-BP=4E-binding protein, **AML**=acute myeloid leukemia, **AP-1**=activator protein-1, **ARNT**=aryl hydrocarbon receptor nuclear translocator, **ATL**=adult T-cell leukemia/lymphoma, **ASXL1**=additional sex combs-like 1, **Bcl-2**=B-cell/CLL lymphoma 2, **BMM**=bone marrow microenvironment, **CA9**=carbonic anhydrase IX, **CAF**=cancer-associated fibroblast, **CBP**=cyclic adenosine monophosphate response element-binding protein, **CK2**=casein kinase 2, **CMMML**=chronic myelomonocytic leukemia, **CXCL**=C-X-C motif chemokine ligand, **DC**=dendritic cell, **ECM**=extracellular matrix, **eIF**=eukaryotic translation initiation factor, **eNOS**=endothelial nitric oxide synthase, **ERK**=extracellular signal-regulated kinase, **FLT3**=feline McDonough sarcoma like tyrosine kinase 3, **FOXO**=Forkhead Box O, **GPCR**=G-protein-coupled receptor, **GSK3 β** =glycogen synthase kinase-3 β , **HAUSP**=herpes virus-associated ubiquitin specific protease, **ITD**=internal tandem

duplication, **HIF**=hypoxia-inducible factor, **HRE**=hypoxia responsive element, **Id1**=inhibitor of DNA binding 1, **IGF**=insulin-like growth factor, **I κ B**=inhibitor of NF- κ B, **IKK**=inhibitor of NF- κ B kinase, **LIC**=leukemia-initiating cell, **LSC**=leukemic stem cell, **MAPK**=mitogen-activated protein kinase, **MDM2**=mouse double minute 2 homolog, **MDSC**=myeloid-derived suppressor cell, **MSC**=mesenchymal stem cell, **mTOR**=mechanistic target of rapamycin, **NEMO**=NF- κ B essential modulator, **NF- κ B**=nuclear factor kappa B, **NK**=natural killer, **NO**=nitric oxide, **NPM1**=nucleophosmin 1, **PDK1**=phosphoinositide-dependent protein kinase 1, **PH**=pleckstrin homology, **PI**=phosphatidylinositol,

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PI3K=phosphatidylinositol 3-kinase, **PR**=protected region, **PTEN**=phosphatase and tensin homolog, **pVHL**=von Hippel-Lindau tumor suppressor protein, **rpS6**=ribosomal protein S6, **RTK**=receptor tyrosine kinase, **RUNX**=runt-related transcription factor, **S6K1**=ribosomal protein S6 kinase 1, **SHIP**=Src homology 2 domain-containing inositol 5-phosphatase, **SP**=specificity protein, **STAT**=transducer and activator of transcription, **TAM**=tumor-associated macrophage, **TGF**=transforming growth factor, **TNF**=tumor necrosis factor, **TSC2**=tuberous sclerosis complex 2, **VEGF**=vascular endothelial growth factor.

INTRODUCTION

Acute myeloid leukemia (AML) is a refractory heterogeneous hematological disease. A small number of leukemic stem cells (LSCs) in the CD34+CD38- AML cells [1-4] are causes of therapy resistance [5,6] and relapse [4]. Thus, elucidation of the mechanisms to generate and induce AML LSCs is quite important and paves the way for development of novel therapies against AML LSCs [1,3,7]. Progression of AML LSCs to overt AML is primarily dependent on cell-intrinsic oncogenic alterations. However, bone marrow microenvironment (BMM) also affects leukemogenesis of AML [1-3,8-10]. In hypoxic BMM, HIF-1 α , HIF-2 α and HIF-3 α are induced [2,9]. In particular, HIF-1 α is involved in leukemogenesis of AML [11-14]. In this concern, aberrantly activated expression of HIF-1 α in AML is quite important for clarification of AML leukemogenesis and development of anti-AML LSC therapies [8]. In addition, activated expression of carbonic anhydrase IX (CA9) is also indicated in AML [15-17]. CA9, which corrects intracellular acidic pH due to glycolysis of cancer cells, promotes cell proliferation, cell survival, metastasis, therapy resistance and tumorigenesis in solid tumors [18-20] and malignant lymphomas [21,22]. Thus, the HIF-1 α /CA9 axis is thought to play an important role in leukemogenesis of AML.

Furthermore, constitutive activation of the phosphatidylinositol 3-kinase (PI3K)/AKT (also known as protein kinase B)/mechanistic target of rapamycin (mTOR) signaling pathway in AML [23,24] has attracted attention with its significance in leukemogenesis and novel molecular targeted therapies [25,26]. Recent studies on a Ser and Thr kinase AKT have revealed that AKT is involved in various diseases, including developmental disorders, malignant diseases, cardiological disorders, diabetes mellitus, inflammatory diseases, autoimmune diseases and neurological disorders [27-30]. However, clarification of the exact mechanisms of its aberrant activation of the PI3K/AKT/mTOR signaling in AML remain challenging issues. In this regard, crosstalk between the PI3K/AKT/mTOR signaling and the HIF-1 α /CA9 axis is crucial, because

many studies have indicated its role in oncogenesis in solid tumors and other hematological malignancies [31-37]. For instance, activation of AKT [38,39], HIF-1 α [38] and CA9 [21] in adult T-cell leukemia (ATL) is confirmed, and the crosstalk between the PI3K/AKT/mTOR signaling and the HIF-1 α /CA9 axis is suggested to be critical for the multistep oncogenesis of ATL [36,37,40,41]. Concerning AML, crosstalk between the PI3K/AKT/mTOR signaling and the HIF-1 α /CA9 axis is pointed out [24], but significance of the crosstalk between the aberrantly activated PI3K/AKT/mTOR signaling and its effectors HIF-1 α /CA9 axis in leukemogenesis of AML has been not well investigated.

This review aims to summarize the aberrant activation of the PI3K/AKT/mTOR signaling pathway and HIF-1 α as well as CA9 in AML and the crosstalk between the PI3K/AKT/mTOR signaling and the HIF-1 α /CA9 axis for clarification of AML leukemogenesis.

REGULATION OF THE PI3K/AKT/mTOR SIGNALING PATHWAY

Activated expression of AKT mRNA and protein is found in many solid tumors [42-44] and hematological malignancies [45] including AML [23-26]. The AKT family consists of three isoforms, AKT1 (AKT α), AKT2 (AKT β) and AKT3 (AKT γ). The upstream regulator of AKT is PI3K. The PI3K family is classified into four classes (class I-IV). Of these, class I PI3K is relevant to signaling activation and oncogenesis [42]. Class I PI3K is a heterodimer and consists of a catalytic α subunit (p100) and a regulatory β subunit (p85, p65, p55 or p100). Class I PI3K is further divided into subclass Ia and subclass Ib. PI3K Ia consists of one of three catalytic α subunits p100 α , p100 β and p100 δ (encoded by *PIK3CA*, *PIK3CB* and *PIK3CD*, respectively) and a regulatory subunit (p85, p65 or p55), while PI3K Ib is consisted of a p100 γ catalytic subunit and a p100 regulatory subunit [42]. When receptor tyrosine kinases (RTKs) or G-protein-coupled receptors (GPCRs) are activated by ligand binding, PI3K is activated. Then, activated PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂) and generates phosphatidylinositol 3,4,5-trisphosphate (PI(3,4,5)P₃) [30]. PI(3,4)P₂ is further generated from PI(3,4,5)P₃ by Src homology 2 domain-containing inositol 5-phosphatase 1 (SHIP1) [46,47]. Mainly PI(3,4,5)P₃ and supplementally PI(3,4)P₂ activate the downstream effectors of the PI3K/AKT/mTOR signaling pathway [27]. In contrast, phosphatase and tensin homolog (PTEN) converts PI(3,4,5)P₃ back to PI(4,5)P₂. Thus PTEN is an inhibitor of the PI3K/AKT/mTOR signaling pathway [27,45,48]. PI(3,4,5)P₃ and PI(3,4)P₂ on the cytosolic side of the plasma membrane recruit proteins that contain pleckstrin homology (PH)

such as AKT and phosphoinositide-dependent protein kinase 1 (PDK1). On the one hand, interaction of PDK1 with PI(3,4,5)P3 by binding of PI(3,4,5)P3 to the PH domain of PDK1 facilitates PDK1-induced activation of AKT [49]. PDK1 itself is activated by PI(3,4,5)P3 [42]. On the other hand, binding of AKT to the PI(3,4,5)P3 on the plasma membrane via the PH domain induces its conformational change for subsequent phosphorylation of the Thr308 and Ser473 residues [50]. Then, Thr308 is phosphorylated by PDK1 [51,52], whereas Ser473 is phosphorylated by mTORC2 [52,53]. mTORC2 is composed of mTOR, Rictor, mLST8, SIN1, Protor and Deptor, while mTOR complex 1 (mTORC1) is composed of mTOR, Raptor, mLST8, PRAS40, FKBP38 and Deptor [23,54].

Activated AKT then activates various downstream signaling pathways including mTORC1 via inhibition of the inhibitor tuberous sclerosis complex 2 (TSC2), Forkhead Box O (FOXO), glycogen synthase kinase-3 β (GSK3 β), inhibitor of nuclear factor kappa B (NF- κ B) kinase (IKK), mouse double minute 2 homolog (MDM2), ribosomal protein S6 kinase 1 (S6K1), and ribosomal protein S6 (rpS6) [25,26,28,42]. These downstream signaling effectors further activate the target molecules that are involved in cell proliferation, cell cycle regulation, cell survival, protein synthesis, and metabolic regulation, leading to oncogenesis of solid tumors [42-44,55] and hematological malignancies [23,24,38,39,45].

ABERRANT ACTIVATION OF THE PI3K/AKT/mTOR SIGNALING PATHWAY IN AML

In solid tumors, genetic mutations associated with the PI3K/AKT/mTOR signaling pathway such as *PIK3CA*, *PTEN*, *PIK3R1*, *PIK3CD* and *AKT1* are frequently found [42], whereas these mutations are not or rarely found in AML [42,56-58]. However, there are several mechanisms to aberrantly activate the PI3K/AKT/mTOR signaling pathway [45,48] (Table 1). First, in spite of no mutation, aberrant expression of PTEN transcripts in 15 of 62 patients (24%) with AML is observed, due to aberrant RNA splicing [56]. This reduces PTEN proteins and leads to activation of PI3K (Table 1). Second, phosphorylation of the C-terminal PTEN at the residues Ser370 and Thr366 by casein kinase 2 (CK2) induces destabilization of the PTEN protein, leading to inactivation of PTEN [59,60]. The C-terminal phosphorylation of PTEN is found in 45 of 61 patients (74%) with AML [61] (Table 1). Third, mutation of nucleophosmin 1 (*NPM1*) is frequently observed in 54 of 200 patients (27%) with AML [58]. Herpes virus-associated ubiquitin specific protease (HAUSP), which is a PTEN deubiquitinating enzyme, induces accumulation of PTEN in the cytoplasm [62], while NPM1 opposes HAUSP by preventing it from its nuclear exclusion of PTEN, and can retain PTEN in the nucleus [63]. Nuclear localization of PTEN is required for its suppressive function. In contrast, the mutated NPM1 does not inhibit the enzymatic activity

Table 1. Aberrant activation of the PI3K/AKT/mTOR signaling pathway.

Mechanisms	Effects	Activated molecules	References
Aberrant expression of PTEN transcripts due to aberrant splicing	Suppression of PTEN	AKT	[56]
C-terminal phosphorylation of PTEN by CK2	Inactivation of PTEN	AKT	[59-61]
Nuclear exclusion of PTEN by HAUSP due to mutated NPM1	Reduction of PTEN	AKT	[62,63]
Activating mutation of <i>FLT3</i>	Activation of mTORC1	4E-BP1, p70S6K, rpS6	[64]
Autocrine/paracrine activation of VEGF/VEGFR-2 signaling	Activation of PI3K and AKT	eNOS	[72]
Autocrine/paracrine activation of VEGF/VEGFR-2 signaling	Activation of PI3K and AKT	Bcl-2	[73]
Autocrine activation of IGF-1/IGF-1R signaling	Activation of PI3K	AKT	[74-76]

Abbreviations: 4E-BP1=4E-binding protein 1, CK2=casein kinase 2, eNOS=endothelial nitric oxide synthase, FLT3=feline McDonough sarcoma like tyrosine kinase 3, HAUSP=herpes virus-associated ubiquitin specific protease, IGF-1=insulin-like growth factor-1, IGF-1R=insulin-like growth factor-1 receptor, mTORC1=mechanistic target of rapamycin complex 1, NPM1=nucleophosmin 1, p70S6K=ribosomal protein 70 S6 kinase (also known as p70-isoform of S6K1), PDK1=phosphoinositide-dependent protein kinase 1, PI3K=phosphatidylinositol 3-kinase, PTEN=phosphatase and tensin homolog, rpS6=ribosomal protein S6, VEGF=vascular endothelial growth factor, VEGFR=vascular endothelial growth factor receptor.

of HAUSP and finally leads to polyubiquitination and degradation of PTEN in the cytoplasm [63]. This aberrant PTEN cellular compartmentalization results in suppression of the PTEN inhibitory activity in the nucleus (Table 1).

Activating mutation of *feline McDonough sarcoma like tyrosine kinase 3 (FLT3)* is frequently found in 56 of 200 patients (28%) with AML [58]. FLT3, a receptor type tyrosine kinase, is also known as CD135. Mutation of *FLT3* is classified into internal tandem duplication (ITD) within the juxtamembrane domain (*FLT3-ITD*) and point mutations within the activation loop of the second tyrosine kinase domain (*FLT3-D835/836*) [64]. These *FLT3* mutations do not activate AKT but activate mTORC1 and its downstream effectors 4E-binding protein 1 (BP1), ribosomal protein 70 S6 kinase (p70S6K, also known as p70-isoform of S6K1) and ribosomal protein S6 (rpS6) [64] (Table 1).

Vascular endothelial growth factor (VEGF) signaling pathway is also associated with the PI3K/AKT/mTOR signaling pathway in apoptosis, cell survival, cell proliferation, angiogenesis and therapy resistance [9,10,65,66]. The VEGF family consists of VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-F and placental growth factor (PGF) [67,68] and its tyrosine kinase receptor include VEGFR-1, VEGFR-2 (also known as kinase-insert domain receptor [KDR] or fetal liver kinase 1 [Flk-1]) and VEGFR-3 (identical with feline McDonough sarcoma like tyrosine kinase 4 [FLT4]) [68]. VEGFR-2 binds VEGF-A, VEGF-C and VEGF-D, while VEGFR-3 binds VEGF-C and VEGF-D [68]. High expression of VEGFR-2 by the autocrine and paracrine mechanisms of VEGF is observed in AML [69], and simultaneous expression of VEGF-C and VEGFR-3 (FLT4) is confirmed in AML [70,71]. On the one hand, the VEGF/VEGFR-2 signaling pathway activates endothelial nitric oxide synthase (eNOS) through activation of the PI3K and AKT. Then, activated eNOS produce nitric oxide (NO), resulting in proliferation of AML cells [72] (Table 1). On the other hand, the VEGF/VEGFR-2 induces B-cell/CLL lymphoma 2 (Bcl-2)-mediated apoptosis suppression via activation of AKT, leading to cell survival in AML [73] (Table 1). In addition, autocrine insulin-like growth factor 1 (IGF-1) from AML cells constitutively activates IGF-1 receptor (IGF-1R) [74]. This autocrine activation of IGF-1/IGF-1R signaling aberrantly activates PI3K and then AKT [75], leading to activation of mTORC1 [76] (Table 1).

REGULATION OF HIF-1A

Induction of AML LSCs and leukemogenesis of AML are affected by combination of genetic alterations, epigenetic dysfunctions and intrinsic/extrinsic factors in BMM [1-3,8-10]. Cellular components of the BMM consist

of LSCs, leukemia-initiating cells (LICs), extracellular matrix (ECM), tumor-associated macrophage (TAM), myeloid-derived suppressor cells (MDSCs), natural killer (NK) cells, dendritic cells (DCs), mesenchymal stem cells (MSCs), osteoblasts, osteoclasts, adipocytes, cancer-associated fibroblasts (CAFs), vascular endothelial cells, Schwann cells, T-lymphocytes and B-lymphocytes [2,9,10], while various non-cellular factors in BMM play relevant roles in promoting LSCs and LICs to overt AML cells, including signaling pathways (PI3K/AKT/mTOR, JAK/transducer and activator of transcription [STAT], RAS/extracellular signal-regulated kinase [ERK], NF- κ B, Hedgehog [HH], WNT/ β -catenin, VEGFR-2, and C-X-C motif chemokine ligand 12 [CXCL12]/CXCR4 signaling), cytokines (interleukin-1 [IL-1], IL-6, tumor necrosis factor- α [TNF- α], and transforming growth factor- β [TGF- β]), metabolic controls (glycolysis, glutaminolysis, and lipogenesis), hypoxia response (HIFs), and acidosis correction (CA9) [1,2,7,9,77,]. Of these, HIF-1 α , HIF-2 α and HIF-3 α that are induced under hypoxic conditions in BMM are important factors, because HIFs activate transcription of numerous target genes and are involved in energy metabolism (glucose transporters [GLUTs] 1/2/3, hexokinases 1/2, phosphofructokinase L, aldolase A, pyruvate kinase M, phosphoglycerate kinase 1, enolase 1 and lactate dehydrogenase A), metabolic adaptation/pH regulation (CA9, CA12), cell proliferation/survival (IGFs, IGF binding proteins 1/2/3, transforming growth factors [TGFs] α/β , epidermal growth factor [EGF] and c-myc), metastasis/invasion (CXCR4 and matrix metalloproteinase 9 [MMP9]), immune escape (programmed cell death protein 1 [PD-1], and PD-1 ligand 1 [PD-L1]), angiogenesis (VEGFs, VEGFs, leptin, LDL-receptor-related protein 1, and adrenomedullin), erythropoiesis (erythropoietin, transferrin, transferrin receptor, and ceruloplasmin), vasomotor control (nitric oxide synthase, adrenomedullin, α 1B-adrenergic receptor and endothelial-1), and therapy resistance [78-82]. Since HIF-1 α , but neither HIF-2 α nor HIF-3 α , activates CA9 [83,84], we focus on HIF-1 α to clarify the roles of the HIF-1 α /CA9 axis in AML leukemogenesis.

Under normoxic conditions in BMM, HIF-1 α in the cytoplasm is subjected to ubiquitin-proteasomal degradation by von Hippel-Lindau tumor suppressor protein (pVHL) [85-87]. In addition, another tumor suppressor protein p53 also promotes Mdm2-mediated ubiquitination and proteasomal degradation of HIF-1 α [88,89]. Conversely, loss of p53 activates expression and function of HIF-1 α [88]. Under hypoxic conditions in BMM, HIF-1 α is quickly induced and accumulates in the cytoplasm due to the inhibition of pVHL-mediated ubiquitin-proteasomal degradation. Then HIF-1 α translocates to the nucleus and forms a

heterodimer with constitutively expressed HIF-1 β (also known as aryl hydrocarbon receptor nuclear translocator [ARNT]). This heterodimer HIF-1 α /HIF-1 β complex directly binds to the hypoxia responsive element (HRE) of target genes [90] with recruitment of cyclic adenosine monophosphate response element-binding protein (CBP)/p300 transcriptional coactivator [91,92]. Factor inhibiting HIF-1 (FIH-1) prevents recruitment of CBP/p300, leading to suppression of HIF-1 α transactivation [87,93,94].

ABERRANT ACTIVATION OF HIF-1A

Mutations of *runt-related transcription factor 1* (*RUNX1*) are found in 20% (19/200) of patients with AML [58] and 37% (30/81) of patients with chronic myelomonocytic leukemia (CMML) [95], while mutations of *additional sex combs-like 1* (*ASXL1*) is often found in CMML patients with

RUNX1 mutations [96]. A significantly high risk of AML transformation is observed in CMML patients with *RUNX1* mutations [95]. *RUNX1*-R135T is involved in aberrant activation of HIF-1 α [96] by direct interaction of the mutated *RUNX1* protein with HIF-1 α protein [97] (Table 2). *RUNX1*-R135T also activates expression of inhibitor of DNA binding 1 (*Id1*). In turn, ID1 promotes activation of AKT [96].

Another mutation *TP53* is observed in 8% (16/200) of patients with AML [58]. Under normoxia, HIF-1 α is controlled by p53-mediated ubiquitin-proteasomal degradation [88,89]. Using HIF-1 α inhibitor echinomycin, Wang et al. [98] demonstrated increased function of HIF-1 α in *TP53*-mutated cell line THP1 and primary cells from patients with *TP53*-mutated AML (Table 2). This indicates activation of HIF-1 α in *TP53*-mutated AML.

Table 2. Aberrant activation of HIF-1 α .

Types of leukemia	Mutations	Mechanisms	References
CMML	<i>RUNX1</i> -R135T	Direct binding to HIF-1 α protein	[96,97]
AML	Mutation of <i>TP53</i>	Inhibition of p53-mediated ubiquitin-proteasomal degradation of HIF-1 α	[88,89,98]

Abbreviations: AML=acute myeloid leukemia, CMML=chronic myelomonocytic leukemia, HIF-1 α =hypoxia-inducible factor 1 α , *RUNX1*=runt-related transcription factor 1.

REGULATION OF CA9 AND ABERRANT ACTIVATION OF CA9 IN AML

In hypoxic BMM, CA9 is induced by binding of the HIF-1 α /HIF-1 β heterodimer complex to HRE in the CA9 promoter with recruitment of CBP/p300 transcriptional coactivator [82,99,100]. There are other binding sites of several enhancer elements in the CA9 promoter [100], i.e., protected region 1 (PR1) and PR5 for binding of specificity protein (SP)-1 and/or SP-3 [101,102], and PR2 for binding of activator protein-1 (AP-1) [101], while PR4 is inhibitory [101].

Even under normoxia, CA9 is activated by aberrantly activated HIF-1 α . Indeed, the HIF-1 α /CA9 axis via its final

effector CA9 plays multiple roles in promoting oncogenic processes of solid tumors and hematological malignancies (18-22,36,37,40), including acidic pH correction [103-105], cell proliferation [18,19,104,105], apoptosis inhibition and cell survival [15,106,107], cell adhesion-invasion and metastasis [108-110], CSC induction and therapy resistance [111,112], epithelial-to-mesenchymal transition (EMT) induction and therapy resistance [113], and tumorigenesis and cell expansion/relapse [21].

Thus the HIF-1 α /CA9 axis is suspected to be crucial also in leukemogenesis of AML. In fact, activated expression of CA9 mRNA in AML is confirmed [17]. In addition, using a CA9/CA12 inhibitor FC531, Chen et al. [16] demonstrated that CA9 is critical for AML cell survival in hypoxic conditions.

Table 3. Promotion of oncogenic processes by the HIF-1 α /CA9 axis in solid tumors and hematological malignancies.

Functions of CA9	Effects	References
Correction of acidic pH	Proliferation	[18,19,103-105]
Inhibition of apoptosis	Survival	[15,106,107]
Cell adhesion and invasion	Metastasis	[108-110]
Induction of CSC	Cancer initiation and therapy resistance	[111,112]
Induction of EMT	Therapy resistance	[113]
Tumorigenesis	Cell expansion and relapse	[21]

Abbreviations: CA9=carbonic anhydrase IX, CSC=cancer stem cell, EMT=epithelial-to-mesenchymal transition, HIF=hypoxia-inducible factor.

CROSSTALK BETWEEN THE PI3K/AKT/mTOR SIGNALING PATHWAY AND THE HIF-1 α /CA9 AXIS IN AML

In general, translation of mRNA [114,115] is activated by signaling pathways such as PI3K/AKT/mTORC1 [116-118] and RAS/ERK signaling [119,120]. In particular, PI3K/AKT/mTORC1 signaling [33,80,121] and RAS/ERK signaling [80] stabilize HIF-1 α protein by translation activation of HIF-1 α mRNA. In addition, NF- κ B activates transcription of HIF-1 α mRNA [122-124]. In this regard, interaction between the aberrantly activated PI3K/AKT/mTORC1 signaling and HIF-1 α is crucial for leukemogenesis of AML by activation of the HIF-1 α /CA9 axis.

On the one hand, activated mTORC1 phosphorylates 4E-BPs. Then phosphorylated 4E-BPs (4E-BP1, -BP2 and -BP3) induce their release from eukaryotic translation initiation factor (eIF4E), leading to the assembly of the mRNA-cap binding eIF4F complex (eIF4E, eIF4G and eIF4A) [114,115,117], and the activated eIF4F complex activates translation of HIF-1 α mRNA [32,33,121]. Activated mTORC1 also activates S6Ks (S6K1 and S6K2). Then activated S6Ks phosphorylate various components of translation and finally activate eIF4B [114,115,117], leading to translation activation of HIF-1 α mRNA [32,33,121]. This is a crosstalk between the PI3K/AKT/mTORC1 signaling and the HIF-

1 α /axis via activation of 4E-BPs and S6Ks, resulting in translation activation of HIF-1 α mRNA. Thus, suppression of PTEN [56,59,60,63], activating mutation of *FLT3* [64], autocrine/paracrine activation of VEGF/VEGFR-2 signaling [72,73] and autocrine activation of IGF-1/IGF-1R [74,76] activate translation of HIF-1 α mRNA and stabilize HIF-1 α protein (Table 4). This activated expression of HIF-1 α protein finally activates CA9.

On the other hand, activated expression of NF- κ B is frequently observed in AML [125-128]. Activated mTORC1 activates IKK [129]. IKK is composed of two catalytic subunits (IKK α and IKK β) and a regulatory subunit (IKK γ , also known as NF- κ B essential modulator [NEMO]). Then, activated IKK phosphorylates I κ B α , leading to its ubiquitin-proteasomal degradation and release of NF- κ B (a heterodimer p50/p65) from inhibition of I κ B α [130,131]. The activated NF- κ B p50/p65 complex translocates to the nucleus and activates transcription of target genes [132]. HIF-1 α is one of the target genes of NF- κ B. The NF- κ B p50/p65 complex binds to the HIF-1 α promoter and activates transcription of HIF-1 α mRNA [122-124]. Thus this is another crosstalk between the PI3K/AKT/mTORC1 signaling and the HIF-1 α /CA9 axis via activation of IKK/NF- κ B, resulting in transcription activation of HIF-1 α mRNA (Table 4). This activated expression of HIF-1 α mRNA and protein also activates CA9.

Table 4. Crosstalk between the PI3K/AKT/mTORC1 signaling pathway and the HIF-1 α /CA9 axis in AML.

Mechanisms	Mediated activation	Activation of HIF-1 α	References
Suppression of PTEN	AKT/mTORC1	Translation of HIF-1 α mRNA	[56,59,60,63]
Activating mutation of <i>FLT3</i>	mTORC1	Translation of HIF-1 α mRNA	[64]
Autocrine/paracrine activation of VEGF/VEGFR-2 signaling	PI3K/AKT/mTORC1	Translation of HIF-1 α mRNA	[72,73]
Autocrine activation of IGF-1/IGF-1R signaling	PI3K/AKT/mTORC1	Translation of HIF-1 α mRNA	[74,76]
Activation of AKT/mTORC1	IKK/NF- κ B	Transcription of HIF-1 α mRNA	[122-124]

Abbreviations: FLT3=feline McDonough sarcoma like tyrosine kinase 3, IGF-1=insulin-like growth factor-1, IGF-1R=insulin-like growth factor-1 receptor; IKK=inhibitor of nuclear factor κ B kinase, mTORC1=mechanistic target of rapamycin complex 1, NF- κ B=nuclear factor κ B, PI3K=phosphatidylinositol 3-kinase, PTEN=phosphatase and tensin homolog, VEGF=vascular endothelial growth factor, EGFR=vascular endothelial growth factor receptor.

These complicated mechanisms of the crosstalk between the PI3K/AKT/mTORC1 signaling and the HIF-1 α /CA9 axis have facilitated research on novel therapies against AML cells, targeting the effectors in the PI3K/AKT/mTORC1 signaling [24,25] and in the HIF-1 α /CA9 axis [16,80,98,133]. However, it is noteworthy that in solid tumors, focus of the research of molecular target therapy in this crosstalk is placed upon the development of anti-CA9 therapies [134-137], because one of the most important

final effectors of the crosstalk between the PI3K/AKT/mTORC1 signaling and the HIF-1 α /CA9 axis is CA9.

CONCLUSION

Aberrant activation of PI3K/AKT/mTORC1 signaling that is induced by suppression of PTEN, activating mutation of *FLT3*, autocrine/paracrine activation of VEGF/VEGFR-2 signaling and autocrine activation of IGF-1/IGF-1R signaling activates translation of HIF-1 α mRNA via

activation of 4E-BPs and S6Ks, and transcription of HIF-1 α mRNA via activation of IKK/NF- κ B. These mechanisms of the crosstalk between the PI3K/AKT/mTORC1 signaling and the HIF-1 α /CA9 axis activate CA9, because the final effector of this crosstalk is CA9. Further research on this crosstalk between the PI3K/AKT/mTORC1 signaling and the HIF-1 α /CA9 axis is required for development of novel therapies against AML LSCs.

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