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Will targeting PI3K/Akt/mTOR signaling work in hematopoietic malignancies?

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Abstract: The constitutive activation of phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) signaling pathway has been demonstrated to be critical in clinical cancer patients as well as in laboratory cancer models including hematological malignancies. Great efforts have been made to develop inhibitors targeting this pathway in hematological malignancies but so far the efficacies of these inhibitors were not as good as expected. By analyzing existing literatures and datasets available, we found that mutations of genes in the pathway only constitute a very small subset of hematological malignancies. Deep understanding of the function of gene, the pathway and/or its regulators, and the cellular response to inhibitors, may help us design better drugs targeting the hematological malignancies.

Keywords: Phosphatidylinositol 3-kinase (PI3K); protein kinase B (Akt); mammalian target of rapamycin (mTOR); hematological malignancies; genetic analysis

Introduction

The components of the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway are frequently mutated in human cancers. This pathway controls the cell cycle, survival, metabolism, and genomic instability of cells that are also the main hallmarks of cancer abnormality (1). Activated PI3K/Akt/mTOR signaling is frequently elicited by the direct mutational activation along the pathway, amplification of genes encoding key components such as *PIK3CA* (2-8), *AKT1* (9) or the loss of tumor suppressor such as *PTEN* (10-13). Many drugs targeting this pathway, alone or in combination with other existing or developing drugs, are currently in clinical trials both for solid tumors and hematologic neoplasms with mediocre efficacies. Here in

this mini-review, we will examine the pathway components and their functions, assess the available clinical and experimental data, and discuss the possible direction we may undergo in developing targeting drugs.

Components of the PI3K/Akt/mTOR signaling pathway and cancer

PI3Ks and PTEN

In mammals, the PI3K family consists of three lipid kinase classes: class I, II and III. Class I PI3Ks is further divided into two subclasses: subclass IA (*PIK3CA*, *PIK3CB* and *PIK3CG*) and subclasses IB (*PIK3CD*). Class I PI3Ks are heterodimers that consist of a catalytic and a regulatory subunit. There are four catalytic isoforms (*PIK3CA*,

PIK3CB, *PIK3CG* and *PIK3CD*) and several regulatory subunits (*PIK3R1*, *PIK3R2*, *PIK3R3*, *PIK3R5* and *PIK3R6*) in class I PI3Ks. Among the four catalytic isoforms of class I PI3K, only *PIK3CA* is frequently mutated in more than 30% of various human solid tumors (14). Mutations of *PIK3CB*, the ubiquitously expressed gene, are rarely seen (15). Class I PI3K regulatory subunits such as *PIK3R1* and *PIK3R2*, were found in cancer cells and resulted in PI3K activation (16,17). Class II PI3Ks are not well defined at present (18). Class III PI3K refers to vacuolar protein sorting 34 (VPS34: PIK3C3), which is a critical regulator of autophagy (19). The phosphatase and tensin homolog deleted on chromosome 10 (PTEN), is the first phosphatase identified as a tumor suppressor. *PTEN* is frequently silenced or mutated in almost all cancers (20).

PDK1 (PDKP1) and Akt

3-phosphoinositide-dependent protein kinase 1 (PDK1 or PDKP1) and protein kinase B (Akt), which belong to serine/threonine (Ser/Thr) kinase, are both members of the AGC kinase family (cAMP-dependent, cGMP-dependent and protein kinase C) (21,22). PDK1 overexpression was observed in breast carcinoma (23), prostate cancer (24), esophageal squamous cell carcinoma (25), melanoma (26), and AML (27), and correlated with tumor invasion and poor prognosis (28). The three main isoforms of Akt are termed Akt1, Akt2, and Akt3. (22). Akt1 was overexpressed in primary human gastric adenocarcinoma (29), Akt2 was overexpressed in ovarian, gastric, breast and pancreatic cancer (30-32). Akt3 was overexpressed in primary melanomas, ovarian tumors, and prostate cancers (33-35). Somatic mutations in Akt1 occurred in a small number of human breast, ovarian, and colorectal cancers (36). Increased copy number of chromosomal region 1q44 (where *AKT3* is located) was observed in hepatocellular carcinomas and glioblastomas (37,38).

FoxOs

The FoxO transcription factors are considered as tumor suppressors that belong to the forkhead box family of transcription regulators (39). The FoxO family contains four members: FoxO1, FoxO3, FoxO4 and FoxO6 (40). The FoxOs are involved in multiple signaling pathways and play critical roles in a variety of biological processes including cell-cycle arrest, differentiation, apoptosis, metabolism and stress resistance (41).

TSC complex and Rheb

The TSC complex, comprising of TSC1, TSC2 and TBC1D7, is GTPase-activating proteins (GAPs) of Ras homologue enriched in brain (Rheb) (42-44). Tuberous sclerosis (TSC), which is caused by mutation of *TSC1* (encoding hamartin) or *TSC2* (encoding tuberin) genes, is a rare genetic disorder characterized by tumor formation in multiple organs (45). Two Rheb family members, Rheb1and Rheb2 (also known as RhebL1) have been identified in mammals. Rheb1 was overexpressed in various cancers, such as non-small cell lung cancer, liver cancer, bladder cancer, breast cancer, head and neck cancers, prostate cancer, and acute myeloid leukemia (AML) (46-52). The human cancer genome data analysis confirmed existence of recurrent Rheb1 mutations (53). Rheb1 deletion augmented the apoptosis of AML cells, and rapamycin combined with Rheb1 deletion further increased AML cell apoptosis in a MLL-AF9 induced mouse model (51).

mTOR

mTOR is an evolutionarily conserved Ser/Thr kinase that senses and responds to various signals to regulate cell growth, cell survival and other multiple biological processes in eukaryotes (54). It belongs to PI3K-related kinases (PI3KKs) (55). mTOR interacts with multi-proteins to form two distinct complexes, designated mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). Raptor and PRAS40 belong specifically to mTORC1, while Rictor, mSin1 and Protor are specific components of mTORC2 (56,57). mTOR complexes play critical roles in both normal hematopoiesis and leukemogenesis (58,59). mTOR hyper-activation was observed in various cancers of 123 individual studies in the cBioportal (http://www.cbioportal.org/, data not shown).

Interactions of these components

Transmembrane tyrosine kinase growth factor receptors (TKGFR) such as insulin-like growth factor 1 receptor (IGF1R), ErbB family receptors and fibroblast growth factor receptors (FGFRs), and G-protein-coupled receptors (GPCR) can initiate the PI3K/Akt/mTOR signaling pathway upon receiving stimulation cues. The activated PI3K is then translocated to the plasma membrane, resulting in the phosphorylation of PIP_2 to PIP_3. PTEN, a negative regulator of this process, dephosphorylates
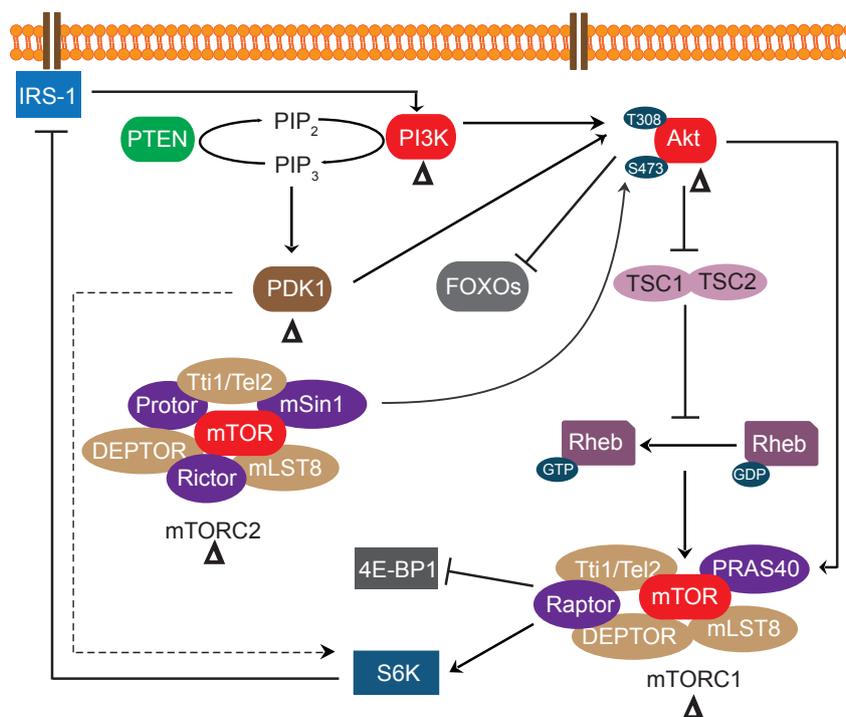
Figure 1 The PI3K/Akt/mTOR signaling pathway and the existing molecular targets for therapy, which was indicated by Δ.

PIP$_3$ to PIP$_2$. Subsequently, PIP$_3$ activates PDK1 and Akt. The activated PDK1 then phosphorylates Akt at T308 and the activated mTORC2 phosphorylated Akt at S473. The activated Akt phosphorylates TSC2, which inhibits TSC1-TSC2 complex. As a GAP for Rheb, TSC1-TSC2 complex can convert Rheb from its active GTP-binding form to inactive GDP-binding form through GTP hydrolysis. Following the inhibition of TSC1-TSC2 complex, Rheb activates mTORC1, leading to protein synthesis and cell growth via 4E-BP1 and S6K1 (*Figure 1*). The phosphorylated S6K1 inhibits the insulin receptor substrate-1 (IRS-1), which promotes IRS-1 degradation and reduces the PI3K signaling to finish the feedback loop (60) (*Figure 1*).

Inhibitors targeting PI3K/Akt/mTOR signaling pathway for hematologic malignancies

The PI3K/Akt/mTOR signaling pathway has been demonstrated to be frequently dysregulated in hematologic malignancies and associated with poor outcome. The constitutive activation of PI3K/Akt/mTOR signaling was observed in 50-80% of AML cases (61-63) and in 87.5% of T cell-acute lymphoblastic leukemia (T-ALL) cases (64).

The pathway was also activated in chronic myelocytic leukemia (CML) (65), chronic lymphocytic leukemia (CLL) (66), multiple myeloma (MM) (67,68), and high-risk myelodysplastic syndrome (MDS) (69). Therefore, much effort has been devoted to developing precision medicine to this pathway for treating hematological malignancies. There are currently four main classes of inhibitors that target the PI3K/Akt/mTOR signaling pathway: PI3K inhibitors, Akt inhibitors, mTOR inhibitors and dual PI3K-mTOR inhibitors (70). PI3K inhibitors consist of pan-PI3K inhibitors and isoform-selective inhibitors. The former are capable of inhibiting all four isoforms of Class I PI3K, while the latter specifically target only one isoform of PI3K (71). Next-generation inhibitor that could overcome mTOR resistance mutations has also been developed (72).

Fransecky *et al*. reviewed the preclinical and clinical trials of these aforementioned inhibitors in AML, B-ALL, B-CLL, T-ALL, non-Hodgkin lymphoma (NHL), and MDS, but found that the clinical trial data are rather disappointing (73). In addition to the four main inhibitors that targeting the PI3K/Akt/mTOR signaling pathway, many other inhibitors are also in development. Studies showed that PDK1 inhibitors could inhibit human AML cell growth *in vitro* (27,74,75). This is supported by

Table 1 The data sets used and the percentages of altered cases in these datasets obtained from the cBioportal

Study abbreviation	Study name	No. of cases	No. of cases altered	Percent cases altered (%)
AML (TCGA)	Acute myeloid leukemia (TCGA, Provisional)	191	8	4.3
AML (TCGA pub)	Acute myeloid leukemia (TCGA, NEJM 2013)	188	8	4.2
MM (Broad)	Multiple myeloma (Broad, Cancer Cell 2014)	205	4	2.0
ALL (St. Jude)	Infant MLL-rearranged acute lymphoblastic leukemia (St Jude, Nat Genet 2015)	24	0	0
DLBC (TCGA)	Lymphoid neoplasm diffuse large B-cell lymphoma (TCGA, Provisional)	48	23	47.9
PCNSL (Mayo Clinic)	Primary central nervous system lymphoma (Mayo Clinic, Clin Cancer Res 2015)	10	0	0

experimental evidence that PDK1 deletion in mice could prolong the survival of MLL-AF9 induced AML mice when compared with the control (76). Interestingly, the dual PI3K/PDK1 inhibitors were more cytotoxic to T-ALL cell lines and primary patients leukemia cell samples when compared with the pan-PI3K inhibitor, Akt inhibitor, mTORC1 inhibitors, and mTORC1/mTORC2 inhibitors used in the same study (77). While so many inhibitors targeting this pathway have been tested, the clinical trial results are rather mediocre in contrast to the convincing preclinical data with the same drugs.
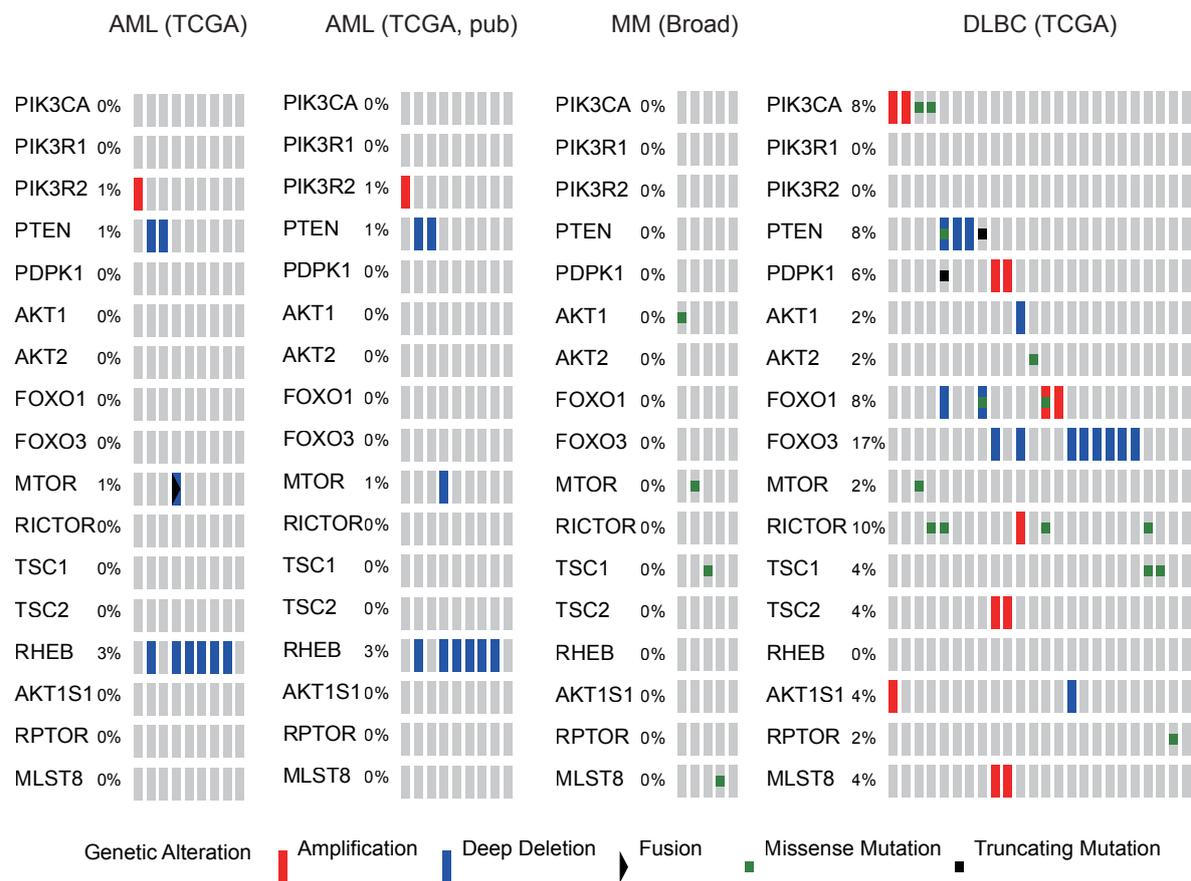
Genetic alterations of PI3K/Akt/mTOR signaling pathway in hematologic malignancies

Why the efficacy of inhibitors targeting this pathway was not as good as expected in contrast to the initial experimental anti-malignancy results? There are three possible interpretations. First, molecules in this pathway have crosstalk(s) and/or feedback loop(s). For example, suppression of mTORC1 inhibits downstream S6K1, inducing IRS-1-dependent negative feedback that promotes activation of PI3K/Akt (60,78). Second, inhibitors developed currently could not totally abrogate the full function of a particular targeted molecule, and the downstream molecules are not always completely inhibited. For example, phosphorylation of 4E-BP1 was not much affected by rapalogs or mTORC1 inhibitors in AMLs (79). Furthermore, cellular metabolic status also affect the inhibition result as Medvetz and colleagues demonstrated that mTORC1 hyper-activation generates metabolic vulnerabilities that can be therapeutically targeted while mTORC1 inhibition alleviates these metabolic

vulnerabilities (80). Third, drug-resistance mutations may occur quickly upon mTOR inhibitor usage in breast cancer cell line (72). Given these, it is essential to further explore the molecular signatures of PI3K/Akt/mTOR in hematological malignancies in order to better the design of inhibitors.

Previous studies showed that the constitutive activation of the PI3K/Akt/mTOR signaling pathway was due to existing mutations of FLT3 in AML (63), BCR-ABL fusion protein in CML (65), and K-RAS/N-RAS mutations or PTEN mutations in MM (67,68). To further explore the dysregulations of this pathway in hematologic neoplasms, we analyzed the mutation types and copy number alterations (CNA) of PI3K/Akt/mTOR signaling pathway genes in hematologic malignancies using the cBioportal for Cancer Genomics (http://www.cbioportal.org/), which provides large-scale cancer genomics data sets. The Cancer Genome Atlas (TCGA) which includes data of AML (from TCGA Provisional, 200 cases and TCGA, NEJM, 2013, 200 cases), Infant MLL-rearranged acute lymphoblastic leukemia (St Jude, Nat Genet 2015, 24 cases), MM (Broad, Cancer Cell 2014, 205 cases), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC, TCGA, Provisional, 48 cases), and primary central nervous system lymphoma (PCNSL, Mayo Clinic, Clin Cancer Res, 2015, 10 cases), were used for mutations analysis (81). We found that the percentages of genetically altered cases in the AML (TCGA), AML (TCGA, pub), MM (broad) and DLBC (TCGA) were 4.3%, 4.2%, 2% and 47.9% respectively, while those of ALL (St. Jude) and PCNSL (Mayo Clinic) were both 0% (*Table 1*).

We then analyzed the mutation types of the 17 selected genes in the PI3K/Akt/mTOR signaling pathway in

Figure 2 Genetic alterations of the indicated PI3K/Akt/mTOR signaling pathway genes in hematological malignancies.

these cases with mutations. In AML (TCGA), there are 1% *PIK3R2* amplification, 1% *PTEN* deep deletion, 1% *MTOR* fusion and missense mutation and 3% *RHEB* deep deletion. In AML (TCGA, pub), there are 1% *PIK3R2* amplification, 1% *PTEN* deep deletion, 1% *MTOR* deep deletion and 3% *RHEB* deep deletion. While there are missense mutations of *AKT1*, *MTOR*, *TSC1* and *MLST8* in MM (Broad), *PTEN* deletions and hotspot mutations of *PIK3CA* and *AKT1* are absent in this MM dataset (82,83). Surprisingly, more mutations are seen in DLBC (TCGA) that involved at least 14 genes in the PI3K/Akt/mTOR signaling pathway (*Figure 2*). Among these detected hematological malignancies, *PIK3R2* amplification and *PTEN* deep deletion in AML, as well as *PIK3CA*, *PDPK1*, *MLST8* and *RICTOR* amplification in DBLC could contribute to the activation of this pathway. However, they only constitute a small subset of hematological malignancies except for DLBC dataset.

Subsequently, we compared the expression of the 17 selected genes in the PI3K/Akt/mTOR signaling pathway in the hematological malignancies VS normal counterparts existed in Oncomine (www.oncomine.org), which is a cancer microarray database that provides gene expression profiles. We found that the expression of *PIK3R1*, *MTOR* and *RHEB* in leukemia, that of *PDPK1*, *AKT2*, *MTOR2*, *RHEB*, *AKT1S1* and *MLST8* in lymphoma, and that of *PI3CA*, *PIK3R2*, *RICTOR* and *AKT1S1* in myeloma were all increased when compared with their normal controls (P<0.05, fold-change ≥2), consistent with the activation of the PI3K/Akt/mTOR signaling pathway in hematological diseases. Similarly, Cancer outlier profile analysis (COPA) showed increased *MLST8* expression in a small subset of lymphoma (4/27) and enhanced *RICTOR* expression in a small subset of myeloma (2/11) (P<0.05, fold-change ≥2) (*Table 2*). Therefore, inhibitors targeting PI3K/mTOR/Akt signaling pathway could inhibit cancer cell growth in a very small proportion of patients with hematological malignancies.

Table 2 Oncomine analysis of gene expression profiles of the PI3K/Akt/mTOR signaling pathway in hematologic malignancies obtained from publicly available microarray datasets

t-test (FC=2; P<0.05)	PIK3CA	PIK3R1	PIK3R2	PTEN	PDPK1	AKT1	AKT2	FOXO1	FOXO3A	MTOR	RICTOR	TSC1	TSC2	RHEB	AKT1S1	RPTOR	MLST8
Cancer vs. normal																	
Leukemia																	
Up	0	2	0	0	1	1	2	5	0	1	0	0	0	1	0	0	0
Down	3	1	0	1	4	2	2	6	3	0	0	1	1	0	0	0	0
Lymphoma																	
Up	0	0	0	1	1	1	3	0	0	0	0	0	0	3	4	0	1
Down	6	0	0	1	0	6	0	13	8	0	6	4	0	0	0	0	0
Myeloma																	
Up	1	0	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0
Down	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
COPA (top 10%) (outlier)																	
Leukemia																	
Up	6 [55]	6 [54]	6 [54]	12 [56]	11 [53]	3 [54]	19 [53]	11 [55]	11 [53]	5 [53]	5 [24]	6 [55]	1 [20]	17 [54]	5 [24]	3 [23]	3 [43]
Down	10 [55]	9 [55]	9 [54]	25 [56]	25 [53]	9 [54]	23 [53]	20 [55]	8 [53]	13 [53]	5 [24]	9 [55]	6 [20]	26 [54]	6 [24]	5 [23]	9 [43]
Lymphoma																	
Up	5 [34]	2 [35]	8 [33]	7 [33]	7 [32]	3 [35]	13 [35]	6 [34]	2 [28]	4 [32]	7 [23]	3 [31]	2 [12]	11 [31]	2 [20]	1 [20]	4 [27]
Down	6 [34]	5 [35]	6 [33]	19 [33]	18 [32]	4 [35]	15 [35]	8 [34]	6 [28]	5 [32]	8 [23]	4 [31]	5 [12]	14 [31]	7 [20]	1 [20]	2 [27]
Myeloma																	
Up	0 [16]	3 [16]	3 [16]	3 [16]	1 [15]	1 [16]	4 [16]	3 [16]	2 [15]	2 [16]	2 [11]	0 [16]	0 [exclusively available]	8 [16]	0 [11]	4 [11]	0 [14]
Down	1 [16]	3 [16]	3 [16]	6 [16]	4 [15]	3 [16]	9 [16]	8 [16]	4 [15]	2 [16]	1 [11]	2 [16]	0 [exclusively available]	4 [16]	2 [11]	2 [11]	2 [14]

The top part shows expression level of indicated genes. Student's t-test was used for all datasets. P<0.05, fold-change ≥2. The lower part shows the number of cases of indicated genes in the datasets analyzed by Cancer Outlier Profile Analysis (COPA, top 10%).

Conclusions and perspectives

Mutations in genes from PI3K/mTOR/Akt pathway resulting in the activation of this pathway comprise only a very small subset of hematological malignancies. Inhibitors to the specific gene products in the pathway failed to show high efficacy in all hematological malignancies thus far. This is probably caused by the remaining activity of the PI3K/Akt/mTOR pathway owing to partial efficacy of the inhibitors, the upregulation of regulatory feedback loop, or the adaptive mutation in response to inhibitors. In view of this, the search for new type of inhibitors that are more effective, precisely targeted and less toxic in the PI3K/Akt/mTOR signaling pathway is urgently needed. More importantly, gene products that regulate the up- or down-stream of the PI3K/Akt/mTOR signaling pathway may provide an alternative for consideration when designing drugs targeting hematological malignancies. In this case, aiming AMPK and MAPK pathways could be an attractive approach. In addition, gene expression is also regulated by additional factors such as epigenetic elements, microRNAs or lncRNAs, thus searching epigenetic changes that regulate these pathways in hematological malignancies may also expand our horizon for curing diseases.

Although the mutation type and expression level of genes in PI3K/AKT/mTOR signaling were widely accessible online, the profiles for their protein expression level data were not available due to probably the lack of such data. Since the protein level is considered to be the most valuable data to corroborate its gene expression level and provide more valid information for doctors to choose specific inhibitors to treat diseases, we would strongly recommend that when possible, protein arrays be performed together with expression array and/or mutation analysis for patients with hematological malignancy. Additionally, microRNA, lncRNAs and epigenetic profiling of leukemic cells will also provide different perspectives for the specific characteristics of a particular evolving disease. Such technologies have been developed and now are widely accessible and affordable with less patient material. We believe that the accumulation of such data will help researchers to better design targeted drugs and clinicians to better treat patients with unique malignancy signature.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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