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The role of exosomes and MYC in therapy resistance of acute myeloid leukemia: Challenges and opportunities

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Abstract

Acute myeloid leukemia (AML) is caused by abnormal production of white blood cells, red blood cells or platelets. The leukemia cells communicate with their microenvironment through nano-vesicle exosomes that are 30–100 nm in diameter. These nano-vesicles are released from body fluids upon fusion of an endocytic compartment with the cell membrane. Exosomes function as cargo to deliver signaling molecules to distant cells. This allows cross-talk between hematopoietic cells and other distant target cell environments. Exosomes support leukemia growth by acting as messengers between tumor cells and the microenvironment as well as inducing oncogenic factors such as c-Myc. Exosomes have also been used as biomarkers in the clinical diagnosis of leukemia. Glycogen synthase kinase-3 (GSK-3) and protein phosphatase 2A (PP2A) are two crucial signaling molecules involved in the AML pathogenesis and MYC stability. GSK-3 is a serine/threonine protein kinase that coordinates with over 40 different proteins during physiological/pathological conditions in blood cells. The dysregulation in GSK-3 has been reported during hematological malignancies. GSK-3 acts as a tumor suppressor by targeting c-MYC, MCL-1 and β -catenin. Conversely, GSK-3 can also act as tumor promoter in some instances. The pharmacological modulators of GSK-3 such as ABT-869, 6-Bromoindirubin-3'-oxime (BIO),

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GS-87 and LY2090314 have shown promise in the treatment of hematological malignancy. PP2A is a heterotrimeric serine/threonine phosphatase involved in the regulation of hematological malignancy. PP2A-activating drugs (PADs) can effectively antagonize leukemogenesis. The discovery of exosomes, kinase inhibitors and phosphatase activators have provided new hope to the leukemia patients. This review discusses the role of exosomes, GSK-3 and PP2A in the pathogenesis of leukemia. We provide evidence from both preclinical and clinical studies.

Keywords

Acute myeloid leukemia; Exosome; Myc; GSK-3; PP2A; Tumor microenvironment

1. Introduction

Leukemia is a type of hematological malignancy caused due to the excessive production of abnormal white blood cells. In 2017, Leukemia represented 30.4 percent of blood cancers, and 2.9 percent of other cancers in the USA (Buckley et al., 2018). Broadly, leukemia can be of two types: acute and chronic. Leukemia is further classified into acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), and other less common types (Vardiman et al., 2009). ALL constitute one of the most common blood cancers worldwide. Although 90 percent of leukemias were diagnosed in adults, three-fourths of pediatric blood cancers are ALL (Disease et al., 2016). The etiology of leukemias are not very clear, but chemicals, smoking, ionizing radiation and family history are some of the common factors. The environmental disturbances and inheritance are the common risk factors associated with leukemia (Juliussen and Hough, 2016; Dahl and Wiemels, 2015).

The chemotherapy, radiotherapy, bone marrow transplantation and palliative care are some of the common treatment methods for leukemia (Dombret and Gardin, 2016). Most often, combination of these therapies is used for therapy. However, the success of the therapy depends on the patient age and the leukemia type. The majority of the treatment regimens fail to respond due to the communication between AML cells and the surrounding bone marrow microenvironment where normal hematopoietic stem cells reside and differentiate into the other blood cell types. The main challenge to the existing leukemia therapy is that the leukemia cells get shelter in the bone marrow environment where they exchange the signals for self-renewal and the development of chemoresistance (Behrmann et al., 2018; Kumar et al., 2018; Wang and Zhong, 2018). Further, the bone marrow niche is also involved in maintaining the stemness of leukemia cells. The bone marrow environment can provide a sanctuary to leukemia stem cells leading to the development of therapy resistance (Holyoake and Vetrie, 2017; Zhou et al., 2016).

Exosomes are extracellular vesicles with 30–100 nm in diameter. Originally identified during the maturation of sheep reticulocytes (Johnstone et al., 1987; Pan et al., 1985), exosomes are now reported to be secreted by many cells including mast cells, dendritic cells, endothelial cells, adipocytes, lymphocytes, neuronal cells and epithelial cells during the cellular homeostasis. These small vesicles are also found in multiple biological fluids such

as serum, plasma, urine, cerebro spinal fluid, tears, milk, etc. (He et al., 2018; Ludwig and Giebel, 2012). Exosomes mediate immune response, differentiation, migration, cell-cell communication, invasion, and angiogenesis (Bobrie and Thery, 2013). Exosomes act as antigen-presenting molecules and can transport antigenic peptides to dendritic cells to strengthen immune response in the target cells (Raposo et al., 1996). The functions of exosomes in the biology of many human cancers including AML is well documented (Ludwig and Giebel, 2012; Kalluri, 2016; Li and Nabet, 2019; Wortzel et al., 2019). Furthermore, novel role of exosomes in upregulating the oncogenic c-Myc have also been studied.

c-Myc is an essential transcription factor that plays a cogent role in leukemogenesis. The *in vitro* and *in vivo* studies have suggested that the c-Myc higher expression correlates with the progression of AML (Luo et al., 2005; Salvatori et al., 2011). The regulation of this transcription factor is tightly controlled during cellular homeostasis. The two key regulators of c-Myc are glycogen synthase kinase 3 β (GSK-3 β) and protein phosphatase 2A (PP2A). These regulators act through the phosphorylation and dephosphorylation events leading to stabilization/destabilization of the transcription factor (Arnold and Sears, 2006; Kazi et al., 2018; Liu and Eisenman, 2012; Vervoorts et al., 2006; Mumby, 2007; Westermarck and Hahn, 2008; Low et al., 2014). GSK-3 β is involved in the regulation of cell metabolism, cell differentiation, apoptosis, autophagy, and tumorigenesis of leukemia (Biver et al., 2014). The GSK-3 β also plays a role in the survival, chemotherapy resistance, and maintenance of stemness in leukemia (Banerji et al., 2012). PP2A is a serine/threonine phosphatase that suppresses the tumor growth and regulates kinase-driven intracellular signaling pathways (Mumby, 2007; Westermarck and Hahn, 2008; Low et al., 2014). The inactivation of PP2A has been reported in various solid tumors and hematological malignancy. This leads to sustained survival and repressed apoptosis (Perrotti and Neviani, 2013; Neviani et al., 2013).

In this review, we discuss exosomes role in drug resistance, leukemic microenvironment, immune response, bone marrow microenvironment, and endothelial dysfunction. We also provide evidence on the potential functions of cMyc and their regulatory components in the progression and therapy resistance of AML. Finally, we emphasize the potential of exosomes as novel therapeutic molecules in the treatment of relapsed or drug-resistant AML patients.

2. Mechanisms of drug resistance in AML

2.1. Exosomes

Exosomes are nanovesicles with 30–100nm in diameter. These are secreted from most cell types of the body fluids. Exosomes mediate a wide variety of pathophysiological events such as differentiation, proliferation and apoptosis in the recipient cells (Skog et al., 2008). Exosomes transport a wide range of components including messenger RNA, microRNAs (miRNAs), long non-coding RNAs, proteins, molecular chaperones, cholesterol, sphingomyelin, ceramide, phosphatidylserine, tetraspanins, and Fas ligand. The exosomes are identified in different stages of biogenesis by using several markers such as ALIX, CD63, CD81, CD9, syndecan-1, tumor susceptibility gene 101 (TSG 101), major

histocompatibility complex (MHC) molecules, and heat shock protein 70 (HSP 70) (Subra et al., 2010).

Recent study established AML derived exosomes mediated immune suppression in patient-derived xenograft mice model (Hong et al., 2019). The leukemia-derived exosomes are known to induce regulatory T cells (Tregs) and polarization of macrophages. Tregs are a subset of heterogeneous CD4⁺ T cell population (Lei et al., 2015). These cells play crucial role in immune homeostasis and can regulate various inflammatory processes such as autoimmunity and tissue injury (Grindebacke et al., 2009; Burzyn et al., 2013). The exosomes are also known to interfere with the cellular differentiation of immune cells for which several mechanisms have been proposed. For example, exosomes can inhibit the differentiation of DCs from the precursor cells (Yu et al., 2007). Exosomes can also trigger the development of TGFβ-producing MSCs (Chowdhury et al., 2015). The Fas-positive exosomes can trigger death in T cells by binding to Fas-ligand (Anel et al., 2019). In the following section, we discuss the potential of leukemia derived exosomes in immune response, endothelial cells, bone marrow microenvironment and drug resistance (Figs. 1–2 and Table 1).

2.1.1. Exosomes and leukemia progression—A high level of exosomes is reported in the biological fluids of cancer patients (Melo et al., 2015). Cancer-derived exosomes maintain tumor microenvironment and mediate several signal transduction pathways that are linked to tumor cell aggressiveness and drug resistance. The elevated levels of exosomes is significantly reduced after chemotherapy in AML patients (Hong et al., 2014). Additionally, hemorrhagic complications before chemotherapy increases the exosomes levels in the patient plasma. Therefore, the altered exosomes could contribute to human leukemia malignancy (Zwicker et al., 2009).

The exosomes released from AML cells contain nucleophosmin 1 (NPM 1), FMS-like tyrosine kinase 3 (FLT 3), matrix metalloproteinase 9 (MMP 9), insulin-like growth factor 1 receptor (IGF-1R), and CXCR 4 (Huan et al., 2013). These molecules participate in the pathogenesis of AML and can serve as potential prognostic biomarkers. In one study, the exosome trafficking from AML cells altered the proliferative, migratory, and angiogenic responses of cocultured stromal and hematopoietic progenitor cell lines (Huan et al., 2013). Furthermore, several AML associated coding and noncoding RNAs were observed in the exosomes. The exosomes can also facilitate leukemic growth by modulating CXCR4–CXCL12 signaling (Schroeder and DiPersio, 2012). These observations further suggest exosomes functions as mediator of leukemia progression.

In mice, melanoma-derived exosomes are also transferred into the bone marrow microenvironment. Subsequently, this induces pre-malignant phenotype in the bone marrow progenitor cells and creates a pre-metastatic niche (Peinado et al., 2012). Peinado et al. (2012) showed that cancer exosomes can modify the bone marrow microenvironment like the tumorigenesis model. MET is a hepatocyte growth factor receptor or tyrosine kinase receptor essential for wound healing, organogenesis, and embryonic development. The exosome-mediated MET was transported to bone marrow progenitor cells from tumor cells. This study provides substantial evidence that melanoma-derived exosomes are essential in

creating a tumor. HSCs and stromal cells maturity are influenced by blast-derived exosome in the bone marrow microenvironment.

The B-cell chronic lymphocytic leukemia (B-CLL) patients derived exosomes can activate AKT/m-TOR/p70S6K/HIF-1 α axis in CLL-BMSCs (Ghosh et al., 2010). These exosomes also induce VEGF production, a survival factor for CLL B cells (Ghosh et al., 2010). The exosomes also regulated the β -catenin pathway and enhanced the expression of c-MYC and cyclin D1 in bone marrow mesenchymal stem cell (BMSC). Overall, this study provides evidence that CLL exosomes can reshape the bone marrow microenvironment towards malignant progression. The *in vitro* and *in vivo* studies also suggest that the exosomes secreted from CML cells can stimulate human BMSCs to produce IL-8 resulting in enhanced growth and survival of leukemia cells (Corrado et al., 2014). Importantly, the CML-BMSC cross-talk was required for maintaining the malignant phenotype (Corrado et al., 2014). The AML-derived exosomes are taken by BMSC and can alter the secretion of growth factors (Huan et al., 2013). This re-programs the stromal microenvironment and favor leukemic blasts (Huan et al., 2013). The exosomes can induce malignant phenotype by transferring the genetic information to normal hematopoietic cells from malignant cells (Hong et al., 2017). When mononuclear cells-derived from regular transplants were co-incubated with K562 BCR-ABL1-positive exosomes, the leukemia-like phenotype was observed both *in vitro* and *in vivo* (Zhu et al., 2014). Furthermore, genomic instability, an induction in the cytidine deaminase activity and reactive oxygen species generation leading to DNA breaks, DNA hypermethylation and upregulation of methyltransferases was observed in the recipient cells (Zhu et al., 2014).

In one study, multiple myeloma bone marrow mesenchymal stromal cells (MM BM-MSCs) derived exosomes could transfer to MM cells (Roccaro et al., 2013). This resulted in the modulation of *in vivo* tumor growth. The miR-15a tumor suppressor was lower in the MM than the normal BM-MSCs. Conversely, the levels of adhesion molecules, cytokines and oncogenic proteins was high in the MM BM-MSC-derived exosomes in comparison to those derived from normal BM-MSCs. Whereas exosomes from MM BM-MSC enhanced tumor growth, those from normal BM-MSC suppressed the growth. Overall, this study provides a mechanistic insight by which exosomes promote tumor growth (Roccaro et al., 2013).

The AML derived exosomes are also reported to play a role in RNA trafficking (Huan et al., 2013). In one study, several coding and non-coding RNAs were reported in the AML derived exosomes. These exosomes were relevant to AML pathogenesis. Furthermore, evidence was provided for the canonical functions of the transferred RNA. The AML exosomes are also known to regulate bone marrow function. For example, AML exosomes are known to induce leukemic invasion of the bone marrow either directly or indirectly through stromal components (Huan et al., 2015). The AML derived exosomes can suppress the functions of hematopoietic stem and progenitor cell (HSPC) (Hornick et al., 2016). This is mediated through stromal reprogramming of niche retention factors. The AML exosome-directed miRNA trafficking to HSPCs can also lead to the systemic loss of hematopoietic function (Hornick et al., 2016). The AML derived exosomes are known to transform bone marrow niche into leukemia permissive environment by modulating multiple molecules such as Rab27a, DKK1, CXCL12, KITL and IGF1 (Kumar et al., 2018). AML derived circulating

exosomes can also deliver immunosuppressive cargos to the recipient cells thereby suppressing the antitumor activities (Hong et al., 2017).

2.1.2. Exosomes and endothelial cells dysfunction—Leukemia derived exosomes can alter the functions of endothelial cells. Umezu et al. (2013) discovered that leukemia cells promote angiogenesis through the transfer of exosomal miRNAs into endothelial cells (Umezu et al., 2014). The authors co-cultured K562 cells with human umbilical vein endothelial cells (HUVECs). The co-culture of HUVECs with K562 cells was found to remarkably upregulate miR-17–92 cluster. The levels of miR-17–92 is high in K562 cells whereas significantly low in HUVECs demonstrating that K562 cells transfer miRNAs into HUVECs through exosomes. Further, the authors visualized the transfer of K562 cells derived exosomal miR-92a, a miRNA from miR-17–92 cluster into HUVECs. At first, precursor form of miR-92a was labeled with Cy3 and transfected to K562 cells followed by co-culture with HUVECs. Red color intensity of Cy3 labeled miR-92a was co-localized with CD63, an exosomal protein marker in the HUVECs cytoplasm. That the miR-92 is transferred through exosomes was further confirmed using GW4869, which is an inhibitor of exosome secretion (Umezu et al., 2014). The K562 cells were co-cultured with HUVECs after transfection with Cy3-labeled pre-miR-92a. The labeled miRNAs were transferred to HUVEC cytoplasm and the Cy3 signal co-localized with CD63 (Umezu et al., 2014). K562/Cy3-miR-92a cells were exposed to GW4869, exosomes were isolated and co-cultured with HUVECs. The Cy3-miR-92a signal was not observed in the HUVEC cells. Further, GW4869 suppressed the exosomal miRNAs secretion by inhibiting ceramide bio-synthesis (Umezu et al., 2013). Another group reported the secretion of exosomes enriched with miRNA-135b from the MM cells (Umezu et al., 2014). In endothelial cells, the miRNA135b-tagged exosomes interact with factor inhibiting hypoxia-inducible factor 1 (FIH-1). This increases the tube formation under hypoxic condition via hypoxia-inducible factor-FIH signaling (Umezu et al., 2014).

Paggetti et al. (2015) demonstrated that stromal cells are transformed into cancer-associated fibroblasts (CAFs) by CLL-derived exosomes (Paggetti et al., 2015). This facilitate the proliferation and inflammatory cytokine secretion. Moreover, leukemic exosomes were found to enhance angiogenesis characteristics in the endothelial cells (Paggetti et al., 2015). Overall, leukemia-derived exosomes transfer miRNA in to endothelial and stromal cells leading to increased angiogenesis. The leukemia-modified stromal cell supports leukemia proliferation and inhibits normal hematopoiesis.

LAMA 84 CML cells-derived exosomes were fluorescently labeled and internalized into HUVECs. These exosomes modify the tubular differentiation, loss of β -catenin and E-cadherin increasing cell motility in the HUVECs (Taverna et al., 2012). CML exosomes-induced growth of murine endothelial cells and Matrigel plugs vascularization were associated with src activation in HUVECs. The leukemic exosome effect was abrogated by the tyrosine kinase inhibitor (Mineo et al., 2012). This study emphasized that leukemic cellsderived exosomes directly affect the endothelial cells and modulate the neovascularization. Therefore, exosomes may play a vital role in tumor progression by modifying the tumor microenvironment.

2.1.3. Exosomes and drug resistance—The inhibition of fibroblast growth factor receptor (FGFR) signaling reduces exosome secretion and abrogate resistance to tyrosine kinase inhibitors (TKIs) in AML (Javidi-Sharifi et al., 2019). Exosomes are also known to protect AML cells from apoptosis in response to chemotherapy (Chen et al., 2019; Wojtuszkiewicz et al., 2016). BMSC exosomes protect from drug mediated apoptosis and induces resistance to bortezomib, which is a proteasomal inhibitor. The BMSC derived-exosomes significantly modulate c-Jun, p53, p38, AKT, and N-terminal kinase in MM cells (Wang et al., 2014). The exosomes derived from BMSC can modulate the tumor growth (Roccaro et al., 2013), angiogenesis (Mineo et al., 2012), and inhibit the induction of anti-tumor NK cell immunity (Reiners et al., 2013). The exosomes from CLL plasma contain miRNAs such as miRNA29, miRNA150, miRNA155, and miRNA223 which are associated with reduced disease outcome (Yeh et al., 2015). The exosomal miRNA150 and miRNA155 can increase with B-cell receptor (BCR) activation. The BCR inactivation by ibrutinib substantially reduces the exosome levels in the CLL patients plasma (Yeh et al., 2015). It can be concluded that leukemia-derived exosomes are the vital component in drug resistance and act as an indicator of the disease burden.

2.1.4. Exosomes as cancer biomarkers—Accumulating evidence suggest that exosomes can be used as biomarker in the AML diagnosis. The biogenesis of cellular exosomes starts with the inward budding of the endosome membrane. These initial intraluminal vesicles consist of the endoplasmic reticulum and components such as clathrin and plasma membrane (Kowal et al., 2014). Multivesicular bodies (MVBs) are late endosomes, characterized by the presence of lumen in multiple exosomes. It fuses with the plasma membrane and is released into the extracellular space as nano-sized membranous vesicles in an ATP-dependent manner (Subra et al., 2010; Kowal et al., 2014). Its molecular cargo contains surface membrane glycoprotein of parental origin (Subra et al., 2010; Kowal et al., 2014). The endosomal sorting complex responsible for transport (ESCRT) enact a leading role in regulating the biological processes such as cellular abscission, MVB biogenesis and viral budding. It is also required in the transport of vesicle content to the lysosome for degradation. This ubiquitin-tagged endosome protein enters organelles via the formation of vesicles. This process is vital to destroy misfolded and damaged proteins (Piper and Katzmann, 2007). Tetraspanin-enriched microdomains (TEMs) contain tetraspanins that perform a pivotal role in the biogenesis of the exosome, protein and nucleic acid sorting and fusion of MVBs with membrane surface producer cells (Andreu and Yanez-Mo, 2014). The cytoskeleton associated with molecular motors such as myosins, kinesins, and molecular switches such as Rab GTPases, are molecules responsible for the fusion with the plasma membrane. The soluble NSF attachment protein receptor (SNARE) is an essential molecular component involved in exosome cargo release process in the target cell. The manner in which the SNARE complex releases exosomes is still not clear (Colombo et al., 2014).

Specific membrane profile molecules present on different endosomes during various stages of synthesis resemble their parental cell characteristics. Thus, exosomes could be used as putative non-invasive biomarker. Because exosomes are derived from body fluids, their molecular profile could predict the pathophysiological status of an individual (Skog et al., 2008; Whiteside, 2018, 2019).

2.2. Tumor microenvironment

The microenvironment is essential not only in the initiation but also in the progression of leukemia. Transplantation studies demonstrated that the disease originates from the microenvironment (Walkley et al., 2007). The mouse osteoprogenitor cells deficient in *Dicer1*, an enzyme involved in microRNA biogenesis culminate in to myelodysplasia and eventually progresses into AML (Raaijmakers et al., 2010). Schwachman-Bodian-Diamond Syndrome- α is a condition in which bone marrow does not function properly and the production of white blood cells is impaired. The osteoprogenitor cells deficient in *Dicer1* exhibited reduced expression of the *Sbds*, a gene frequently mutated in Schwachman-Bodian-Diamond Syndrome- α (Raaijmakers et al., 2010). The β -catenin activating mutation in mouse osteoblasts can lead to leukemia (Kode et al., 2014). Further, the Notch ligand jagged 1 expression is stimulated by the activated β -catenin in osteoblasts. Interestingly, the intervention of Notch signaling ameliorates acute myeloid leukemia thus confirming the pathogenic role of this pathway (Kode et al., 2014). Overall, microenvironment contribute to the leukemia development. Conversely, leukemia may also induce changes in the tissue microenvironment that helps in further progression of the disease.

During leukemia, alterations in the hematopoietic stem cells (HSCs) niche has also been reported. These alterations support the disease progression. The leukemia stem cells (LSC) are restricted to cells with long-term hematopoietic stem cell (LTHSC) phenotype (Zhang et al., 2012). A reduction in the homing and retention in the bone marrow (BM) was demonstrated by the CML LTHSC. This relates with the suppressed CXCL12 expression in CML BM and increased production of GCSF in leukemia cells. Furthermore, altered cytokine production in CML BM was correlated with selective impairment of normal LTHSC growth and a growth advantage to CML LTHSC. The abnormalities in cytokine levels and LTHSC growth was partially corrected by the use of imatinib (Zhang et al., 2012).

The myeloproliferative neoplasia (MPN) progressively remodels a leukemic niche that favors bone marrow fibrosis and leukemic stem cell function (Schepers et al., 2013). The authors found that the multipotent stromal cells are stimulated by the leukemic myeloid cells. This results in the production of excessive and altered osteoblastic lineage cells that accumulate in the bone marrow cavity. The group identified the roles of thrombopoietin, transforming growth factor- β (TGF- β), Notch, chemokine (C-C motif) ligand 3 (CCL3) and inflammatory signaling in osteoblastic lineage cell remodeling. MPN expanded the osteoblastic lineage cells to display the deteriorating expression of the HSCs retention factors and to compromise the ability of the cells to maintain the healthy HSCs and support leukemia stem cells (Schepers et al., 2013). The normal osteoblast population plays vital role in preventing the leukemia progression.

The patient-derived mesenchymal stromal cells are vital for the propagation of myelodysplastic syndrome-initiating lineage such as CD34 and CD38 stem cells in orthotopic xenografts (Medyouf et al., 2014). The factors such as N-cadherin, vascular endothelial growth factor α (VEGF α), insulin-like growth factor binding protein 2 (IGFBP2), and leukemia inhibitory factor (LIF) are associated with patient-derived mesenchymal stromal cells to enhance myelodysplastic syndrome expansion. Hematopoietic-stromal interactions are necessary for these niche factors in myelodysplastic

syndrome. These factors could be used as therapeutic targets for disrupting hematopoietic stromal interactions in myelodysplastic syndrome. The normal mesenchymal stem cells significantly adopt myelodysplastic syndrome mesenchymal-like features when exposed to hematopoietic myelodysplastic syndrome cells which indicate instructive remodeling of the environment (Medyouf et al., 2014).

The changes in the tumor microenvironment may help the leukemic cells to resist to anti-leukemic drugs. The functions of bone marrow microenvironment in the leukemia chemoresistance is well documented by several investigators. The stromal cells of the niche could provide a protective environment for AML, ALL and CLL (Bendall et al., 1994; Manabe et al., 1992; Panayiotidis et al., 1996). The stromal cells can alleviate the apoptosis of leukemia cells by releasing anti-apoptotic factors. During the chemoresistance stage, AML cells produce very late antigen-4 (VLA-4). The VLA-4 binds to the fibronectin component of stromal niche and aid in the anchorage of leukemia cells leading to development of drug resistance (Matsunaga et al., 2003). The VLA-4 negative patients have significantly higher five-year survival rate as compared to VLA-4 positive patients. Further, CD44 contribute to leukemic cell engraftment and maintains homeostasis in AML and CML mice models (Mumby, 2007). The antibody blockage of CD44 reduces the disease burden. The leukemia cell survival depends on their direct contact with the bone marrow microenvironment (Jin et al., 2006; Krause et al., 2006).

In mice xenograft model, the chemoresistant primary human AML cells are engrafted near to endosteal region of the bone marrow microenvironment (Ishikawa et al., 2007). In these cells, cytotoxic drugs induce apoptosis at a lower level as compared to AML cells present in the center of bone marrow. Thus, the growth of AML cells can be supported by their localization and adhesion to the microenvironment (Ishikawa et al., 2007).

The microenvironment of endothelial cells also supports the proliferation and survival of leukemia cells. For example, T cell acute lymphoblastic leukemia (T-ALL) are reported to be in direct contact with CXCL12-producing bone marrow stroma (Pitt et al., 2015). Furthermore, the deletion of CXCL12 from vascular endothelial, but not perivascular cells can impede tumor growth. The genetic targeting of Cxcr4 in murine T-ALL after disease onset reduced the disease phenotype in a rapid and sustained manner. Also, the human T-ALL in primary xenografts was suppressed by antagonizing CXCR4. The loss of CXCR4 was associated with decreased leukemia initiating cell activity *in vivo* by targeting key T-ALL regulators (Pitt et al., 2015).

From the above discussion, it is clear that microenvironment is crucial for leukemogenesis. Thus, targeting the communication between the leukemia cells and endothelial cell microenvironment or inhibiting changes in the microenvironment may be an attractive approach for the leukemia therapy.

The interaction of exosomes with leukemic microenvironment contributes to immune system invasion by leukemic cells. Haderk et al. (2017) discovered that CLL-derived exosomes had abundant RNA species hY4 which were identified in RNA sequencing and compared to healthy donors. CLL-derived exosomes or hY4 alone co-culture with monocytes

significantly promoted CLL-associated phenotypes including the release of CCL2, CCL4, IL6, cytokines and program death-ligand-1 (PD-L1). The CLL-associated phenotypes were abolished by toll-like receptor 7 (TLR 7)-deficient monocytes. It was concluded that inhibition of endosomal TLR signaling considerably reduces monocytes activation (Haderk et al., 2017). The AML cells are known to secrete VEGF/VEGFR-containing exosomes (Wang et al., 2019). In HUVECs, the VEGF/VEGFR-containing exosomes can induce glycolysis leading to vascular remodeling and acquisition of chemoresistance (Wang et al., 2019). AML T cells are functionally impaired, unable to form efficient immune synapses and autologous blasts. This leads to the host immune response failure against leukemic blasts (Le Dieu et al., 2009). The exosomes from the serum of the AML patients can suppress immune functions of the natural killer (NK) cells by reducing their cytotoxicity (Szczepanski et al., 2011). These exosomes contain elevated levels of TGF β 1, a potent immunosuppressor for NK cells and it down-regulated NKG2D, a transmembrane protein/c-type lectin-like receptors expression (Szczepanski et al., 2011). Based on the previous studies, NK cell activities such as cytokine production and intracellular-signaling are lowered in AML. NK cell dysfunction could be a causative factor for initiation, development, progression or relapse of AML (Tajima et al., 1996). Therefore, the understanding of the leukemic microenvironment and exosomes interaction with the immune system may help in the development of promising anti-leukemia immunotherapy.

2.3. Role of cMyc and its regulators in drug resistance

The proto-oncogene c-Myc is a transcription factor of the helix-loop-helix–leucine zipper family of proteins which critically maintains proliferation, differentiation, apoptosis and cell cycle (Raaijmakers et al., 2010). The c-Myc regulation is crucial for preserving cell proliferation in healthy cells. The reduced expression of c-Myc plays an important role in leukemia. The over-expression of c-Myc is observed in most human hematopoietic malignancies (Pelengaris et al., 2002). c-Myc enacts an essential role in the induction of leukemogenesis in AML cells. The expression of c-Myc in many human hematopoietic malignancies correlates with poor prognosis. The amplification of c-Myc is well documented in few leukemia cell lines (Langenau et al., 2003). The expression of c-Myc is tightly regulated at post-transcriptional and translational levels in normal cells. Especially, Ser⁶² and Thr⁵⁸ phosphorylation in the N-terminal part of c-Myc control its stability. Thus, c-Myc destabilization is tightly associated with ubiquitin-mediated proteolysis (Sears et al., 2000).

Upon stimulation with growth signals, c-Myc undergoes phosphorylation at Ser⁶² and Thr⁵⁸ followed by its ubiquitination and degradation (Yeh et al., 2004). The c-Myc phosphorylation at these two sites is regulated by phosphatidylinositol 3-kinase (PI3K) and Raf/MEK/ERK pathways. The Raf/MEK/ERK pathway can improve c-Myc protein stability by ERK-mediated Ser⁶² phosphorylation. The c-Myc protein stability is enhanced by PI3K mediated phosphorylation at Thr⁵⁸ (Gregory et al., 2003). The mutations in FLT3 receptor tyrosine kinase and fusion proteins enhances the c-Myc expression in AML (Gilliland and Griffin, 2002). Further, oncogenes such as PLZF-RARA, PML-RARA, and AML1-ETO promote myeloid leukemogenesis (Muller-Tidow et al., 2004).

2.3.1. GSK3 beta—Glycogen synthase kinase-3 (GSK-3) is a serine/threonine kinase ubiquitous in nature. It is a rate-limiting enzyme in glycogen metabolism and exists in two isoforms, GSK-3 α and GSK-3 β . This kinase regulates a wide variety of cellular processes such as signal transduction and gene expression, and can determine the cell fate (Jope and Johnson, 2004). GSK-3 personate a critical role in variety of human diseases including neurological disorders, type 2 diabetes, stroke, and neoplasm (Cohen and Frame, 2001; Rayasam et al., 2009). GSK-3 reacts with almost 40 different substrates associated with cancer pathogenesis (Luo, 2009; Ougolkov and Billadeau, 2006). GSK-3 β is a vital tumor suppressor and introduces phosphate group to pro-oncogenic molecules such as β -catenin (Rubinfeld et al., 1996), cyclin D1 (Diehl et al., 1998), HSF-1 (Chu et al., 1998), NFATc (Beals et al., 1997), CREB (Tullai et al., 2007), c-Myc (Sears et al., 2000), and c-Jun (de Groot et al., 1993). The phosphorylation of these molecules by GSK-3 β triggers them for ubiquitin mediated proteasomal degradation.

In its tumor suppressive function, GSK-3 β regulates the Wnt signaling to pacify β -catenin's inhibitory phosphorylation and prevents cell cycle progression (Fig. 3 and Table 2). Tumor-promoting pathways activate the β -catenin and stabilize the c-Myc protein to promote the tumorigenesis (Rayasam et al., 2009). Wang et al. (2008) reported that mixed-lineage leukemia (MLL)-associated leukemia depends on GSK-3 activity for the continued proliferation of transformed cells. The pharmacological suppression of GSK-3 decreases cell cycle progression, proliferation, and increases myeloid differentiation of leukemia cells which transform into chimeric MLL oncoproteins. The p27Kip1, a cyclin-dependent kinase inhibitor was eloquently elevated in MLL leukemia cells after treatment with GSK-3 inhibitors (Wang et al., 2008). Wang et al. (2010) divulged that the HOX/MEIS1/CREB complex is responsible for recruiting the co-activators CBP and TORC and maintain the MLL leukemia stem cell transcription (Wang et al., 2010).

The MLL is a histone H3 Lys-4 specific methyltransferase positively regulating Hox expression. HOX gene is continuously expressed in hematopoietic stem and progenitor cells during normal hematopoiesis. MLL fusion proteins significantly influence the aberrant expression of HOX, which enumerate with MEIS1 to maintain leukemia cell transformation. Wang et al. (2010) reported that GSK-3 activates the downstream pathways of MLL fusion proteins to promote HOX-mediated transcription (Wang et al., 2010). Once GSK-3 is inhibited, HOX-transformed cells abate the proliferation, HOX/MEIS1 activity in a CREB dependent manner. The HOX/MEIS1 targeted genes transcription is regulated by these coactivators. The transcriptional activating complex depends on the GSK-3 activity that enhances leukemia stem cells proliferation. GSK-3 inhibitors are promising candidates for MLL associated leukemia transformed cells (Shah and Sukumar, 2010). Caudal-type homeobox transcription factor (CDX2) regulate HOX genes which is expressed in most of the AML cases (Rice and Licht, 2007).

The GSK-3 suppression by lithium chloride has been reported effective in the *in vivo* model. The lithium-ion acts as a non-specific inhibitor to compete with the magnesium for protein binding area that facilitates GSK-3 inhibitors. This complex is a target for GSK-3 activity in MLL, and specific substrate inhibitors may be developed for the HOX/MEIS1/CREB complex in MLL (Shah and Sukumar, 2010; Rice and Licht, 2007). A recent study

postulated that knockdown of GSK-3 isoforms can significantly deplete HSCs. Thus, GSK-3 could be necessary for self-renewal of normal HSCs.

2.3.2. PP2A—Protein phosphatase 2A (PP2A) is a Ser/Thr phosphatase that exists in dimeric and trimeric forms. It acts as a tumor suppressor and reverses most of the kinase-driven intracellular signaling (Mumby, 2007; Westermarck and Hahn, 2008; Low et al., 2014) (Fig. 3 and Table 2). The activity of this phosphatase is significantly suppressed in solid and hematological malignancies. The reduction in the PP2A activity is normally associated with enhanced survival and suppressed apoptosis (Neviani et al., 2013).

The structural mutation in PP2A-A α and PP2A-A β subunits leads to impaired binding of B and C subunits. This inhibits PP2A activity and promote cellular transformation. Downregulation of the A β subunits is commonly observed in most AML patients. c-KIT is a type of stem cell factor receptor or receptor tyrosine kinase, found on cell surfaces bound to stem cell factor. The mutations in c-KIT leads to altered expression of the scaffold protein and changed regulatory subunits in AML patients (Gao et al., 2015). c-KIT promotes differentiation, survival, and proliferation. The mutations in oncogenic c-KIT suppress PP2A activity by reducing the protein levels of PR55 α , PR61 α , PR61 γ , PR61 δ , and PR65 α . An overexpression of PP2A-A α can prevent the c-KIT-mediated growth and survival in myeloid c-KIT + cells. These observations suggest that the drug resistance can be reversed by restoring PP2A activity in c-KIT + AML patients (Roberts et al., 2010).

In one study, the expression of multiple transcripts in newly diagnosed AML patients was quantified (Ruvolo et al., 2011). An elevation in the level of PPP2CA, PPP2CB, and PPP2R2A was observed in the blast cells (Diehl et al., 1998). The PR55 α subunit of PP2A directly links to AKT dephosphorylation (Ruvolo et al., 2011). Thus, the strategies to promote PR55 α and AKT inactivation may be potential avenue for the AML therapy (Ruvolo et al., 2011). In AML, PR55 α which is the PP2A regulatory subunit, is implicated in DNA damage response. PP2A is activated by RNA-activated protein kinase and protein kinase R (PKR) by facilitating the PR55 α /B55 α nuclear localization (Cheng et al., 2015). The PP2A activation antagonized autophosphorylation and supported ATM expression to prevent DNA damage response (Cheng et al., 2015). PKR is a central mediator present in the hematopoietic progenitor cells for the anti-proliferative effects of hematopoietic cellular stresses such as hematopoietic growth factor deprivation, viral infection, TLR ligands, inflammatory cytokines, and chemotherapy treatment. PKR stimulates the PP2A-PR61 α that results in BCL2 dephosphorylation in mitochondria and downregulates PP2A-PR61 β and PP2A-PR61 γ in AML cells. Subsequently, this leads to the PP2A inactivation and contributed to malignant leukemic cell proliferation (Cristobal et al., 2011). The loss of PP2A subunits such as PR61 β /B56 β and PR61 γ /B56 γ could result in AML leukemogenesis. PR61 ϵ /B56 ϵ is down-regulated in AML patients, contributing to cell proliferation. An impairment in cell proliferation, induction in caspase-dependent apoptosis, modulation in the AKT activation, and reduction in the colony-forming ability of the leukemic cells was observed by PR61 ϵ (Cristobal et al., 2013). This suggest that PR61 ϵ downregulation is mediated partly through p53 (Cristobal et al., 2013).

3. Chemo sensitization of AML

The drugs based on GSK3 and PP2A can be used for the chemosensitization of leukemia cells. However, a number of immunological barriers hamper leukemia treatment (Table 3). The utility of GSK3 and PP2A based drugs in the leukemia therapy is discussed in the following section.

3.1. GSK3 inhibitors

GSK3 regulates diverse pathways that may affect leukemogenesis such as survival, chemotherapy resistance, leukemia stem cell growth and autophagy in CML cells. However, autophagy via tyrosine kinase inhibitor or imatinib (Drullion et al., 2012). The GSK3 α/β phosphorylation was analyzed in 511 AML patients by reverse phase protein analysis (Ruvolo et al., 2015). While a positive correlation was observed between p-GSK3 α/β and p-AKT, P70S6K, and BAD; the p-GSK3 α/β was negatively correlated with FOXO3 α and β -catenin. The group identified that AKT-mediated GSK3 α/β phosphorylation might be required for the AML survival. So, p-GSK3 α/β may be promising prognostic factor for a subset of AML patients.

Tyrosine kinase inhibitors are crucial for leukemia therapy. Mutations in the FMS-like tyrosine kinase 3 (FLT3) gene are frequently observed in AML. The attractive target to activating mutations such as FLT3, c-KIT, MEK, NPM1, IDH1 and IDH2 are important therapeutic targets in AML cells (Grunwald and Levis, 2015). The deletion of GSK-3 β in HSCs produced a pre-neoplastic state in consistence with human myelodysplastic syndromes. The GSK-3 α and GSK-3 β contributed to AML by modulating the Wnt/Akt/mTOR signaling. GSK-3 β could also be used as a prognostic marker for the disease progression and the MDS patient survival after the deletion of the molecular signature of HSCs. Overall, GSK-3 α and GSK-3 β -associated pathways could help for transformation to MDS and subsequent AML. These pathways are identified as potential therapeutic targets (Guezguez et al., 2016). The role of GSK3 in regulating resistance to quizartinib (AC220), an inhibitor of FLT3, was examined in AML patients (Hou et al., 2017). The FGF/Ras/ERK and Wnt pathways were involved in the development of resistance to AC220 by AML patients. The SPRY3 and GSK-3 α expression was suppressed in AC220-resistant AML patients. Further, primary AML cells deficient in SPRY3 were resistant to AC220.

GSK3 supports the pro-survival molecules such as c-MYC, MCL-1 and β -catenin. However, the potential of GSK as a therapeutic target depends on the particular cancer type. One potential strategy for the treatment of leukemia is to suppress PI3K/AKT pathway thereby activating GSK3 (McCubrey et al., 2014). ABT-869, the multi-kinase inhibitor suppresses AKT activation while inducing GSK3 activation (Hernandez-Davies et al., 2011). PI3K inhibitor (BEZ235) with BH3 mimetic can suppress the expression of BCL-2 and BCL-XL in AML cell lines (Rahmani et al., 2013). MCL-1 is a crucial target of GSK in AML cells (Rahmani et al., 2013). The pharmacological inhibition or genetic knockdown of GSK-3 can prevent MCL-1 reduction. A study depicted that GSK-3 acts as a tumor promoter in leukemia, and is involved in the resistance of AML cells to daunorubicin (De Toni et al., 2006).

The pre-clinical studies have also demonstrated promise of GSK3 inhibitors in AML, ALL, and CML (Song et al., 2010). In the *in vitro* and *in vivo* leukemia models, 6-bromoindirubin-30oxime (BIO) has shown efficacy. The differentiation can be induced in AML cells by GS-87 which is a specific inhibitor of GSK3 (Hu et al., 2016). The efficacy of LY2090314, which is a GSK3 inhibitor was tested in AML patients in a Phase II clinical trial (Rizzieri et al., 2016). GSK-3 inhibition was observed in the patients. Thus, the drug could produce potential benefits when combined with other medications.

3.2. PP2A-related drugs

FTY720 is a sphingosine analog derived from *Isaria sinclairii*. Approved for the therapy of hematological tumors, this drug acts as a potent PP2A activator in leukemias (Oaks et al., 2013). The activated PP2A induces apoptosis by interfering with BCL2 and reduces the mitogenic and survival signals by suppressing the PI3K/AKT and ERK pathways (Chen et al., 2014). FTY720 produces effects in both positive and negative leukemias. FTY720 supports the BCR-ABL1 inactivation and degradation in Ph-positive B-ALL and CML progenitor cells which lead to inhibition of survival factors. CD34⁺ progenitors in patients with TKI-resistant and TKI sensitive CML undergo apoptosis in response to FTY720 (Neviani et al., 2007).

SET proteins are potent endogenous inhibitor of PP2A in myeloid leukemias. SET was identified in acute undifferentiated leukemia as oncogene fused with nucleoporin NUP214 (Li et al., 1996). It is involved in DNA repair, DNA replication, differentiation, chromatin remodeling, gene transcription and cell cycle regulation (Kandilci et al., 2004). The role of SET in CML was extensively studied. An over-expression in SET was reported in CML through BCR-ABL1, the oncogenic tyrosine kinase that is essential for CML maintenance, development, and progression (Neviani et al., 2005).

FTY720 suppresses the sphingolipid metabolism. This results in the accumulation of pro-apoptotic second messenger ceramide in the mitochondrial membrane and supports the AML cells death (Neviani et al., 2007). FTY720 containing lipid nanoparticles increased the oral bioavailability of the free drug. It is considered as an oral therapeutic drug for AML (Estella-Hermoso de Mendoza et al., 2015). Thus, PP2A could be considered as a novel target for AML. It acts as a regulator of survival and proliferation pathways that are often activated in AML leading to activation of oncogenic kinases.

4. Exosome therapy for AML: is it possible?

The therapeutic potential of exosomes for leukemia-related malignancy has been demonstrated by a number of studies. MSC-derived exosomes can compel the mitigated patient's peripheral blood mononuclear cells, pro-inflammatory cytokine response and improved clinical graft-versus-host disease symptoms shortly after the start of MSC-derived exosome therapy (Kordelas et al., 2014). Exosomes are capable of efficiently delivering drugs, miRNAs, and antigens to target cells in hematological malignancies. Exosomes containing siRNAs can also cross the blood-brain barrier (Wahlgren et al., 2012). This property of exosomes could be utilized for the treatment of CNS-associated leukemias that poorly respond to chemotherapy. Exosomes accumulation in plasma is linked to venous

thrombolytic events in hematological malignancies (Zwicker et al., 2009). Exosomes carry tissue factors and could interact with the coagulation cascade. The large numbers of procoagulant vesicles in cancer patient's plasma was a worse prognostic factor that was misleading the clinical diagnosis of patients. As a result, efforts have been taken to therapeutically remove or reduce such exosomes in ongoing clinical trials (Zwicker et al., 2013).

Recently, methods are being developed to selectively silence exosome-delivered messages that promote cancer growth. Several approaches are aimed at inhibiting exosome-mediated immune suppression in recipient cells. ALIX, which is a small-molecule inhibitor can attenuate the exosome biogenesis (Baietti et al., 2012). In K562 CML cells, intracellular Ca^{2+} can also regulate exosome secretion (Savina et al., 2003). In mice model of EL4 lymphoma, exosome secretion can be reduced by dimethyl amiloride (DMA), which is a $\text{Na}^+/\text{Ca}^{2+}$ exchange inhibitor. DMA inhibited the mouse and human tumor growth by suppressing the secretion of exosomes carrying HSP70 (Chalmin et al., 2010).

5. Conclusion and future perspectives

It is clear from above discussion that leukemic bone marrow and exosome-mediated signaling contributes to leukemia progression. Exosomes are the vital messengers that regulate leukemia development in recipient cells. The malignant leukemic cells can also develop resistance mechanism through mediation of exosomes. On the other hand, exosomes secreted by normal hematopoietic or tissue cells mediate an anti-tumor response. Exosomes have emerged as sensitive, specific, and non-invasive biomarkers for hematological malignancy. Adverse effects caused by the off-target activity of GSK-3 inhibitors was obtained by the agents that bind to the ATP-competitive binding site. The pharmacological restoration of PP2A tumor-suppressor activity can effectively antagonize leukemogenesis.

Several outstanding questions on the *in vivo* relevance of exosomes to leukemia progression needs to be addressed. The detailed molecular mechanism for the release, segregation, trafficking and uptake of exosomes by recipient leukemic cells should be addressed. More studies should examine the efficacy of GSK-3 and PP2A based drugs in the clinic. Novel approaches are required to reduce the cost and adverse effects of GSK-3 and PP2A inhibitors for AML patients.

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Fig. 1. Schematic representation for the functions of leukemia-derived exosomes in bone marrow microenvironment, immune system, endothelial cells and mesenchymal stem cells.

Leukemia-derived exosomes educate tumorigenesis in bone marrow microenvironment to transport tyrosine kinase receptor MET activating HF-1 α , AKT, VEGF, c-Myc, IL-8 and cyclin D1 pathways. It transports hY4 RNA, TGF β 1 and regulates the CCL2, CCL4, PD-L1 and IL-6 pathways in the immune system. The leukemic exosomes also promote angiogenesis by carrying various microRNAs in endothelial cells. In drug resistant mesenchymal stem cells, leukemic exosomes modulate p53, p38, N-terminal kinase, c-Jun and AKT pathways.

Fig. 2. Effects of leukemia-derived exosomes on immune cells.

Leukemia-derived exosomes induces Treg and polarization of macrophages. Conversely, the exosomes interfere with the cellular differentiation of myeloid progenitor, lymphoid progenitor and dendritic cells, and suppresses the function of NK cells.

Fig. 3. Schematic representation for the role of GSK-3 and PP2A in the leukemia growth. GSK-3 mitigate the cell cycle regulator proteins, activate the HOX/MEIS1 transcription and fusion protein to facilitate the proliferation of MLL cells. It promotes the AKT, BAD, P70S6K pathways and inhibits the β -catenin, FOXO3 α expressions in AML. In CML, GSK-3 regulates the autophagy. PP2A can regulate the c-KIT, PKR, ATM, ERK and PI3K/AKT.

Table 1

Role of leukemia-derived exosomes in various types of leukemia.

Cancer type	Leukemia-derived exosomes functions	Reference
AML	Released CXCR4, FLT3, IGF-1R, MMP9 and NPM1 in to target cells. Downregulated CXCR4 and CXCL12 in Ba/F3 cells	Jin et al. (2006)
CLL	Transported the hY4 RNA species. Induced CLL associated phenotype including production of cytokines, CCL2, CCL4, IL-6 and PD-L1	Ishikawa et al. (2007)
AML	Suppressed NK cells immune function	Skog et al. (2008)
Melanoma	Mediated tyrosine kinase receptor MET from tumor cells to bone marrow progenitor cells	Melo et al. (2015)
B-CLL	Activated AKT, HF-1 α and VEGF in B-CLL cells. Increased cyclin D1 and c-MYC expression in bone marrow mesenchymal stem cells	Hong et al. (2014)
CML	Produced IL-8 in human BMSC	Zwicker et al. (2009)
AML	Altered growth factors secretion in BMSC	Jin et al. (2006)
BCR-ABL1-positive	Induced cytidine deaminase and ROS in mononuclear cells	Huan et al. (2013)
Multiple myeloma	Interacted with miR-135b and FIH-1 in endothelial cells to increase tube formation	Ghosh et al. (2010)
CLL	Promoted proliferation, cytokine secretion and angiogenesis	Corrado et al. (2014)
CML	Modified the tubular differentiation, loss of β -catenin and cadherin in HUVECs	Zhu et al. (2014)
BMSC	Increased the expression of c-Jun, p53, p38, N-terminal kinase, and AKT in MM cells	Umezue et al. (2013)
CLL	Transported miR-223, miR-155, miR-150, and miR-29 family Increased the B-cell receptor activation	Paggetti et al. (2015)

Abbreviations: AML: acute myeloid leukemia; B-CLL: B-cell chronic lymphocytic leukemia; BMSC: bone marrow derived stem cells; CLL: chronic lymphocytic leukemia; CML: chronic myeloid leukemia; CXCL: C-X-C motif chemokine receptors; FLT3: FMS-like tyrosine kinase 3; HIF1: hypoxia-inducible factor 1; HUVEC: human umbilical vein endothelial cell; IGF: insulin-like growth factor; MM: multiple myeloma; MMP 9: matrix metalloproteinase 9; NPM1: nucleophosmin1; PD-L1: programmed death-ligand 1; VEGF: vascular endothelial growth factor.

Table 2

Role of GSK3 and PP2A in various types of leukemia.

Target	Leukemia	Function	Reference
GSK3- β	MLL	Promoted proliferation, cell cycle and myeloid differentiation in leukemic cells	Andreu and Yanez-Mo (2014)
GSK3- β	MLL	Promoted the HOX/MEIS1/CREB complex and activated CBP and TORC	Colombo et al. (2014)
GSK3- β	AML	Regulated CDX2	Langenau et al. (2003)
GSK3- β	CML	Regulated autophagy via tyrosine kinase inhibitor	Yeh et al. (2004)
p-GSK3 α/β	AML	Increased the expression of P70S6K, BAD, and AKT and inhibited FOXO3A and β -catenin expression	Bhatia et al. (1993)
GSK-3 α/β	AML	Modulated the Wnt/Akt/mTOR signaling	Muller-Tidow et al. (2004)
GSK3- β	AML	Increased β -catenin, MCL-1, c-MYC and PI3K/AKT	Cohen and Frame (2001)
GSK3- β	AML	Promoted MCL-1 expression	Luo (2009)
GSK-3 α/β	AML	Induced daunorubicin resistance	Ougolkov and Billadeu (2006)
PP2A	AML	Decreased the activity by c-KIT mutations	Diehl et al. (1998)
PP2A	AML	PR55 α influenced the AKT dephosphorylation	Rubinfield et al. (1996)
PP2A	AML	Promoted ATM expression and prevented DNA damage	Wang et al. (2010)
PP2A	AML	Suppressed cell proliferation, colony-formation and induced caspase-dependent apoptosis	Shah and Sukumar (2010)
PP2A	AML	Induced apoptosis, inhibited ERK and PI3K/AKT pathways	Huang et al. (2009)

Abbreviations: AML: acute myeloid leukemia; CDX2: caudal-type home box transcription factor; c-KIT: tyrosine-protein kinase kit; CML: chronic myeloid leukemia; CREB: cAMP response element binding protein; FOX: fork head family of transcription factors; GSK-3: glycogen synthase kinase-3; MEIS1: meis home box 1; MLL: myelogenous lymphocytic leukemia; mTOR: mammalian target of rapamycin; PP2A: protein phosphatase 2A.

Table 3

A list of immunological barriers for leukemia treatment.

Leukemia	Treatment barrier	Mechanism(s)	Reference
CLL	T cells	Peripheral tolerance	Umezū et al. (2013)
AML	Premature immune response	Inhibition of T cell-induced apoptosis	Umezū et al. (2014)
AML	Defect priming	Defect in the immunogenicity, co-stimulation and dendritic cells maturation	Paggetti et al. (2015)
AML	Immune escape	Defects in MHC expression; immature or loss of antigens	Taverna et al. (2012)
AML	Inhibitory signaling	Regulatory T cells (Tregs)	Mineo et al. (2012)
CLL	Block signaling	PD-1/PD-L1 axis	Tajima et al. (1996)
ALL	Immunosuppressive	IL-10	Le Dieu et al. (2009)

Abbreviations: AML: acute myeloid leukemia; CLL: chronic lymphocytic leukemia; HLA: human leukocyte antigen; IL-10: interleukin-10; MHC: major histocompatibility complex; NK: natural killer; PD-1: programmed death protein-1; PD-L1: programmed death ligand protein-1; TGF-β: transforming growth factor-β.