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Targeting AMPK-ULK1-mediated autophagy for combating BET inhibitor resistance in acute myeloid leukemia stem cells

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ABSTRACT

Bromodomain and extraterminal domain (BET) inhibitors are promising epigenetic agents for the treatment of various subsets of acute myeloid leukemia (AML). However, the resistance of leukemia stem cells (LSCs) to BET inhibitors remains a major challenge. In this study, we evaluated the mechanisms underlying LSC resistance to the BET inhibitor JQ1. We evaluated the levels of apoptosis and macroautophagy/autophagy induced by JQ1 in LSC-like leukemia cell lines and primary $CD34^{+}$ $CD38^{-}$ leukemic blasts obtained from AML cases with normal karyotype without recurrent mutations. JQ1 effectively induced apoptosis in a concentration-dependent manner in JQ1-sensitive AML cells. However, in JQ1-resistant AML LSCs, JQ1 induced little apoptosis and led to upregulation of BECN1/Beclin 1, increased LC3 lipidation, formation of autophagosomes, and downregulation of SQSTM1/p62. Inhibition of autophagy by pharmacological inhibitors or knockdown of BECN1 using specific siRNA enhanced JQ1-induced apoptosis in resistant cells, indicating that prosurvival autophagy occurred in these cells. Independent of MTOR signaling, activation of the AMPK (p-Thr172)-ULK1 (p-Ser555) pathway was found to be associated with JQ1-induced autophagy in resistant cells. AMPK inhibition using the pharmacological inhibitor compound C or by knockdown of PRKAA/AMPKα suppressed autophagy and promoted JQ1-induced apoptosis in AML LSCs. These findings revealed that prosurvival autophagy was one of the mechanisms involved in the resistance of AML LSCs to JQ1. Targeting the AMPK-ULK1 pathway or inhibition of autophagy could be an effective therapeutic strategy for combating resistance to BET inhibitors in AML and other types of cancer.

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AMPK-ULK1; autophagy; BET inhibitor; leukemia stem cells; resistance

Despite advances in our understanding of the molecular pathogenesis of acute myeloid leukemia (AML), the prognosis remains poor, primarily because of high relapse rates. Cytotoxic therapy has shown limited effectiveness in eradicating the functionally distinct leukemia stem cells (LSCs), which may contribute to chemotherapy resistance and relapse. Therefore, novel treatment strategies with acceptable toxicity that target LSCs are urgently needed to improve therapeutic outcomes for patients with AML.

BRD4 (bromodomain containing 4), a member of the bromodomain and extraterminal domain (BET) family, is a chromatin reader that preferentially localizes to "super-enhancer" regions upstream of a variety of oncogenes, including *MYC/c-Myc*, to regulate their expression. BRD4 inhibition may be an effective alternative strategy for targeting *MYC*, which is considered undruggable and deregulated in many human cancers. Selective inhibitors of BET, such as JQ1, are promising epigenetic agents for the treatment of various tumor types, including AML and LSCs.

Although leukemia cells appear to have differences in apoptotic responses to JQ1 and this cannot be explained by changes in the levels of MYC and BRD4, our knowledge of the molecular mechanisms underlying resistance to BET inhibitors remains incomplete. A recently published study suggested that resistance to BET inhibitors in AML is not mediated through abnormal drug efflux or metabolism but emerges from LSCs, and not all LSCs are intrinsically resistant to BET inhibitors. We confirmed that the response differs among LSC-like cells regardless of MYC inhibition, although a substantial proportion of primary AML LSCs is resistant to JQ1. A better understanding of the mechanisms involved in AML LSC resistance to JQ1 is needed to combat resistance to BET inhibitors in AML. Accordingly, we investigated the mechanisms underlying the resistance of LSCs to JQ1.

Anticancer therapies commonly activate prosurvival autophagy, allowing cancer cells to overcome cytotoxic or other stresses induced by the treatment. We described previously the involvement of autophagy induction in the resistance of myeloid leukemia cells to the cytosine arabinoside. Paradoxically, previous studies have reported that MYC activates the unfolded protein response, enhancing cell survival by inducing cytoprotective autophagy; conversely, MYC inhibition decreases autophagy in cancer cells. Autophagy induced by MYC or BET inhibitors has not been examined in cancer cells. Therefore, we

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examined whether JQ1 induced autophagy in the AML LSC candidate cell lines KG1, KG1a, and Kasumi-1, and $CD34^+$-enriched blasts obtained from 13 patients with AML having a normal karyotype. We showed that autophagy is attenuated in JQ1-sensitive LSCs after JQ1 treatment, resulting in apoptosis. Interestingly, we found, for the first time, that cytoprotective autophagy increases the ability of JQ1-resistant LSC-like cell lines and primary $CD34^+$ $CD38^-$ LSCs to resist apoptosis by JQ1. Experiments with autophagy inhibition using knockdown of BECN1 or pharmacological inhibitors confirmed that autophagy induction by JQ1 exposure is a critical mechanism involved in JQ1 resistance in AML LSCs. We demonstrated that 5′ AMP-activated protein kinase (AMPK) activation after JQ1 treatment induces autophagy through ULK1 (unc-51 like autophagy activating kinase 1) phosphorylation at Ser555 in JQ1-resistant LSCs, independent of MTOR (mechanistic target of rapamycin) inhibition. Targeting of AMPK with a pharmacological inhibitor and siRNA decrease JQ1-induced autophagy in LSCs, leading to an increase in JQ1-induced apoptosis. Based on our study, differential expression or mutations in genes associated with AMPK-mediated autophagy induction may serve as a prognostic marker in AML. However, future studies are needed to determine how these cellular processes are differentially regulated between JQ1-sensitive and JQ1-resistant AML LSCs.

In conclusion, the AMPK-ULK1 pathway plays a key role in autophagy of LSCs, conferring resistance to JQ1-induced apoptosis. Moreover, treatment with inhibitors of autophagy or AMPK could overcome JQ1 resistance and enhance the apoptotic response, suggesting the potential clinical utility of these unique targeted therapies against AML LSCs and possibly in other types of cancer.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.