The Chromosome 21 Kinase DYRK1A and Its Substrate FOXO1 Constitute a Novel Therapeutic Pathway in B-ALL

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Abstract

Dual Specificity Tyrosine-Phosphorylation-Regulated Kinase 1A (DYRK1A) is a serine/threonine kinase that regulates diverse pathways such as splicing, cell cycle, differentiation, apoptosis, and transcription. *DYRK1A* is encoded within the Down syndrome (DS) critical region of chromosome 21, underlying its importance in DS-related pathologies, such as Alzheimer's disease. Children with DS have an increased risk of developing hematologic malignancies, namely acute megakaryoblastic leukemia (DS-AMKL) and B-cell acute lymphoblastic leukemia (DS-ALL). We previously reported that *DYRK1A* promotes DS-AMKL by regulating subcellular localization of its substrate NFAT. In a subsequent study, we examined its role in normal hematopoiesis and found that DYRK1A is necessary for B and T cell development through phosphorylation and destabilization of Cyclin D3. *Dyrk1a*-deficient large pre-B cells and double negative thymocytes are unable to enter quiescence for maturation. Despite elevated levels of Cyclin D3, however, these cells lose proliferative capacity due to a block at the G2-M transition. This observation suggests that DYRK1A inhibition may exhibit anti-tumor activity in lymphocytes by first stimulating exit from quiescence but then blocking repeated rounds of cell division.

Notably, *DYRK1A* is overexpressed in acute leukemias, including both T-ALL and B-ALL, relative to normal hematopoietic counterparts. Moreover, overexpression of dominant-negative *DYRK1A-K188R* impairs proliferation in human B-ALL cell lines, suggesting that DYRK1A kinase activity is required for B-ALL growth. In order to assess the physiologic relevance of targeting DYRK1A *in vivo*, we generated a murine model of B-ALL with a floxed *Dyrk1a* allele and observed significant survival advantages with homozygous (p=0.0045) and heterozygous deletion (p=0.0015). Additionally, both B-ALL cell lines and patient samples were sensitive to EHT1610, a potent and selective DYRK1 inhibitor. Relevant to the localization of DYRK1A on chromosome 21, DS-ALL samples were especially sensitive to kinase inhibition. EHT1610 also conferred synergistic growth inhibition of B-ALL cells when combined with cytotoxic chemotherapy drugs used in traditional ALL treatment regimens, such as dexamethasone, methotrexate and cytarabine.

We next aimed to elucidate the mechanism by which DYRK1A inhibition cause a failure of G2-M progression. Using global and directed phosphoproteomic studies, we identified several DYRK1A substrates in pre-B cells that are involved in cell cycle, splicing, transcriptional regulation, and RNA metabolism. In addition to Cyclin D3, a notable substrate is FOXO1, an indispensable transcription factor in B lymphopoiesis. We observed that inhibition of DYRK1A led to an accumulation of FOXO1 in the nucleus of large pre-B cells despite intact PI3K/Akt signaling, which is the predominant negative regulator of FOXO1. Treatment of pre-B cells with AS1842856, an inhibitor of FOXO1 nuclear translocation, rescued the G2-M blockade and proliferative impairment induced by EHT1610 treatment. Despite FOXO1 acting as a tumor suppressor in normal lymphocytes, B-ALL cell lines and patient samples were paradoxically sensitive to FOXO1 inhibition, suggesting a unique requirement in the survival of B-ALL cells. This may be due to regulation of DNA damage, as DYRK1A inhibition alone led to negligible changes in gamma-H2AX foci, whereas FOXO1 inhibition increased DNA damage. When DYRK1A and FOXO1 were inhibited in combination, we observed a synergistic accumulation of DNA damage along with cell death in B-ALL cell lines.

Finally, as both EHT1610 and AS1842856 are potent inhibitors of B-ALL cell growth in vitro, we assessed their *in vivo* efficacy. Both EHT1610 and AS1842856 significantly increased survival in xenograft models of B-ALL (p=0.0002 and p=0.001, respectively). We therefore conclude that both DYRK1A and its substrate FOXO1 are therapeutic targets in B-ALL. Importantly, EHT1610 represents the first selective DYRK1A inhibitor with suitable *in vivo* activity. Ultimately, we have determined that the DYRK1A pathway is integral to the maintenance of normal and malignant B-lymphopoiesis, the latter which can be effectively targeted through 1) a primary proliferative impairment, 2) sensitization to cell cycle-dependent chemotherapy, and 3) downstream inhibition of DYRK1A substrates such as FOXO1.