Inhibition of KSP by ARRY-520 Induces Cell Cycle Block and Cell Death via the Mitochondrial Pathway in AML Cells

Kinesin spindle protein (KSP or Eg5), a microtubule-associated motor protein, plays an important role in establishing a bipolar spindle during mitosis and is essential for cell cycle progression. It has been demonstrated that inhibition of KSP prevents bipolar spindle formation leading to mitotic arrest and cell death. As such, a KSP inhibitor may have anti-tumor potential without toxicities associated with anti-microtubule agents such as taxanes.

We found that KSP is highly expressed in various acute leukemia cells. Inhibition of KSP by a specific inhibitor, ARRY-520, at low nanomolar concentrations, blocked cell cycle progression and led to subsequent cell death in OCI-AML3, Molm13, HL-60, U937, and Jurkat cells. Knocking down p53 by p53shRNA in OCI-AML3 cells did not alter the effectiveness of ARRY-520. U937 cells overexpressing XIAP (U937XIAP) and Jurkat cells lacking caspase 8 (Jurkat I2.1) showed unchanged sensitivities to ARRY-520 compared with their respective control cells, suggesting that cell cycle block and cell death induced by KSP inhibition are independent of p53 status, XIAP levels, and the activation of the extrinsic apoptotic pathway. However, although ARRY-520 blocked cell growth and induced mitotic arrest in both HL-60 cells and HL-60 cells overexpressing Bcl-2 (HL-60Bcl-2), cell death (determined by annexin V staining and changes in mitochondrial potential) was significantly abolished in HL-60Bcl-2 cells. These results suggest that cell death following cell cycle blockade by KSP inhibition is mediated through the intrinsic mitochondrial pathway. Furthermore, ARRY-520 induced the protein level of Bim, a proapoptotic BH3-only Bcl-2 family protein, prior to the activation of caspases in HL-60 cells. Although KSP inhibition by ARRY-520 had no effect on the survival of non-dividing AML blasts in vitro, ARRY-520 significantly inhibited the colony formation capacities of AML blasts, further supporting the critical role of KSP in cell proliferation. Our studies demonstrate that inhibition of KSP by ARRY-520 potently induces blockade of cell cycle progression which leads to cell death of various leukemic cells via the mitochondrial pathway and has the potential to eradicate AML progenitor cells.

Introduction

- Kinesin Spindle Protein (KSP, Eg5) is a member of the kinesin-like protein family and a microtubule-associated motor protein.
- KSP plays a critical role in mitosis by maintaining spindle dynamics and is essential for chromosome positioning and separation, establishing a bipolar spindle, and separating spindles during mitosis.
- KSP is highly expressed in various malignant cells.
- Inhibition of KSP selectively disrupts mitotic spindles in dividing cells and has broad anti-tumor activity while avoiding toxic side effects caused by taxanes and vinca alkaloids.
- ARRY-520 is a specific and potent KSP inhibitor developed by Array BioPharma.

Results

1. KSP is Highly Expressed in Leukemic Cell Lines and in Most Samples of AML Blasts

2. Inhibition of KSP by ARRY-520 Promotes Cell Death in Various Leukemic Cells

3. ARRY-520 Induces G2/M Cell Cycle Block Prior to Cell Death in OCI-AML3 Cells

4. ARRY-520 Induced Cell Death in OCI-AML3 Cells Occurs Primarily in G2/M Cells

5. ARRY-520 Induces p53, but Promotes p53-Independent Cell Cycle Block and Cell Death

6. ARRY-520 Induces Cell Cycle Block and Cell Death in Both U937neo and U937XIAP Cells

7. Jurkat Cells Lacking Caspase-8 (Jurkat I2.1) Are as Sensitive as the Control Jurkat Cells to ARRY-520

8. Overexpression of Bcl-2 Does Not Affect Cell Cycle Arrest but Blocks Cell Death in Response to ARRY-520

9. ARRY-520 Induces Bim Protein Levels in HL-60 Cells Prior to Caspase Activation

10. ARRY-520 Induces Bim Protein Levels in HL-60 Cells Prior to Caspase Activation

Conclusions

- Inhibition of KSP (Eg5) by ARRY-520 blocks cell cycle progression and leads to subsequent cell death in various leukemia cells.
- Cell cycle block and cell death induced by ARRY-520 are independent of p53 status, XIAP levels, and the activation of the extrinsic apoptotic pathways in leukemic cells.
- Inhibition of KSP by ARRY-520 induces cell death via the mitochondrial-mediated pathway.
- ARRY-520 significantly abrogates blast colony forming capacities of samples from AML patients.
- ARRY-520 inhibits growth and promotes death of leukemic cells and has the potential to eradicate AML progenitor cells.

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