Identification of STK33 Kinase Inhibitors for the Synthetic Lethal Relationship Between STK33 and Mutant KRAS

Yihong Zhang, Robert Kurzeja, Anke Munzli, Jim Zondlo, Tisha San Miguel, John Robinson, John McCarter, Ralf Schwandner, Yu Sun, Amro Shehabeldin, Aaron Ellison, Cheryleene Plewa, Ted Judd, Manfred Ferrari, Carol Babij, Josette Carnahan and Isabelle Dussault

ABSTRACT

The inclusion of the serine/threonine kinase STK33 was recently shown to be required for the survival of mutant KRAS cell lines in vitro. In this work, we identified STK33 kinase inhibitors that affect the activity of STK33 in mutant KRAS cell lines. To this end, we identified 1200 small molecules using a high-content screen and tested them in a panel of cell lines. Two hits, 2A and 2B, were selected for further study. In vitro, both compounds inhibited STK33 activity and reduced cell viability in a dose-dependent manner. In vivo, both compounds demonstrated anticancer activity in a xenograft model.

RESULTS

Figure 1. Expression and Purification of Full-Length Active STK32

Figure 2. The pT558K (Thr388) Peptidase is a Substratome for Recombinant STK33

Figure 3. Determination of Enzymatic Properties for Recombinant STK33

Figure 4. Identification of STK33 Kinase Inhibitors by High Throughput Screening

Figure 5. Small Molecular STK33 Kinase Inhibitors Do Not Selectively Affect the Survival of Mutant KRAS-Dependent Cell Lines

Figure 6. No Correlation between Inhibition of STK33 Kinase Activity and Inhibition of RPS6 Phosphorylation in Mutant KRAS-Dependent Cell Lines

INTRODUCTION

STK33 is a serine/threonine kinase with several potential functions, including regulation of the cell cycle, DNA damage response, and epithelial-mesenchymal transition. The expression of STK33 is upregulated in cancer tissues, and its activation is associated with a poor prognosis. The inhibition of STK33 activity may therefore be a potential therapeutic target for cancer treatment.

OBJECTIVE

Identify STK33 kinase inhibitors to test the hypothesis that the STK33 kinase activity regulates RPS6 phosphorylation and that it is required for the survival of mutant KRAS-dependent cell lines.

SUMMARY / CONCLUSIONS

- An active, full-length, heptadecapeptide STK33 kinase was expressed and purified, and its enzymatic properties were determined.
- Over 400,000 compounds were screened with an optimized STK33 kinase assay using a pT558K peptide as a substrate.
- Multiple potent STK33 inhibitors were identified, many with IC50 values less than 100 nM.
- There was no correlation between inhibition of STK33 kinase activity and inhibition of RPS6 phosphorylation in mutant KRAS-dependent mutant KRAS cells.
- There was no correlation between inhibition of STK33 kinase activity and inhibition of cell survival in mutant KRAS-dependent cell lines.

ACKNOWLEDGMENTS

We would like to thank the many team members from Agilent San Francisco, Agilent Thousand Oaks and Agilent Germany that participated in this project.