Inhibiting Glucosylceramide Synthase Sensitizes Chronic Myeloid Leukemia T315I Mutant to Bcr-Abl Inhibitor and Cooperatively Induces Glycogen Synthase Kinase-3-regulated Apoptosis

Inactivation of glycogen synthase kinase-3 (GSK-3) has been implicated in cancer progression. We previously showed anti-CML strategy by accumulating endogenous ceramide to re-activate GSK-3 and abrogate drug resistance. Efficacy was Bcr-Abl-restricted and acted through GSK-3-mediated apoptosis. This study suggests a feasible novel approach to tackle problematic drug-resistant CML T315I mutants to Bcr-Abl inhibitor-induced apoptosis. Combining glucosylceramide synthase inhibitor increased Bcr-Abl-inhibition-induced apoptosis. Furthermore, glucosylceramide synthase inhibitor sensitized the most clinical relevant cell line, primary cells from CML T315I patients to Bcr-Abl inhibitor-induced proliferation inhibition and apoptosis. The synergistic efficacy was Bcr-Abl-restricted and acted through GSK-3-mediated apoptosis. This study suggests a feasible novel anti-CML strategy by accumulating endogenous ceramide to re-activate GSK-3 and abrogate drug resistance.

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Weil-Ching Huang,1,2,3 Cheng-Chien Tsai,1,2,3 Chi-Ling Chen,1 Tsai-Yun Chen,1 Ya-Ping Chen,1,4 Yee-Shin Lin,1 Pei-Jung Lu,1 Chi-Wen Tsao,4 Chi-Yun Wang,1,3 Chi-Yuan Hsiu,1 and Chiou-Feng Lin1,2,3

1Institute of Clinical Medicine, Institute of Basic Medical Sciences, 2Department of Microbiology and Immunology, 3Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng Kung University, Tainan 701, Taiwan; 4Department of Oncology, St. John's Hospital, National Cheng Kung University, Tainan 701, Taiwan

ABSTRACT

Inactivation of glycogen synthase kinase-3 (GSK-3) has been implicated in cancer progression. We previously showed that inactivation of glycogen synthase kinase-3 (GSK-3) is a feasible novel anti-CML strategy by accumulating endogenous ceramide to re-activate GSK-3 and abrogate drug resistance. Efficacy was Bcr-Abl-restricted and acted through GSK-3-mediated apoptosis. This study suggests a feasible novel anti-CML strategy by accumulating endogenous ceramide to re-activate GSK-3 and abrogate drug resistance.

INTRODUCTION

Inactivation of glycogen synthase kinase-3 (GSK-3) has been implicated in cancer progression. We previously showed that inactivation of glycogen synthase kinase-3 (GSK-3) is a feasible novel anti-CML strategy by accumulating endogenous ceramide to re-activate GSK-3 and abrogate drug resistance.

AIMS

- Oncogenic Tyrosine Kinase Bcr-Abl
- Chronic Myeloid Leukemia (CML)
- Glycogen Synthase Kinase-3 (GSK-3)
- Tumor Suppressor Lipid Ceramide
- Glucosylceramide Synthase (GCS)
- Anti-Cancer Drug Resistance
- Bcr-Abl Gatekeeper Mutation T315I

MATERIALS AND METHODS

- Cell culture / Colony forming assay / Proliferation test (WST-8)
- DAPI, Annexin V and PI staining / Western blotting / Flow cytometry
- Transfection / RNA silencing / RNA interference

RESULTS

- FIG. 1. Activating GSK-3 downregulated cell proliferation and augmented Bcr-Abl-inhibitor GNF-2-induced apoptosis.
- FIG. 2. Inhibiting Bcr-Abl activated GSK-3 and induced apoptosis in a Bcr-Abl-dependent manner.
- FIG. 3. Exogenous ceramide or endogenous ceramide accumulation increased GSK-3 activation.
- FIG. 4. Inhibiting GCS by either PDMP or shRNA increased GNF-2-induced GSK-3 activation and apoptosis.
- FIG. 5. GNF-2/PDMP combination overcame GNF-2 resistance in CML T315I patients.

CONCLUSIONS

- GSK-3 negatively regulates the oncogenic activity of Bcr-Abl in chronic myeloid leukemia.
- Combining glucosylceramide synthase inhibitor PDMP with Bcr-Abl inhibitor GNF-2 or imatinib abrogates gatekeeper mutation T315I-directed drug resistance.

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- Institute of Clinical Medicine, Institute of Basic Medical Sciences, Department of Microbiology and Immunology, Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng Kung University, Tainan 701, Taiwan; Department of Oncology, St. John’s Hospital, National Cheng Kung University, Tainan 701, Taiwan

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1. Institute of Clinical Medicine, Institute of Basic Medical Sciences, Department of Microbiology and Immunology, Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng Kung University, Tainan 701, Taiwan; Department of Oncology, St. John’s Hospital, National Cheng Kung University, Tainan 701, Taiwan

FIGURE LEGENDS

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