GCN2 inhibition sensitizes prostate cancer cells to loss of p21 cell cycle control

Haroon M. Mohiuddin[1], Ricardo A. Cordova[1, 2], Noah R. Sommers[1], Angela J. Klunk[1], Kirk A. Staschke[1, 2], Ronald C. Wek[1, 2].

[1]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana, USA.
[2]Indiana University Simon Comprehensive Cancer Center, Indiana University School of Medicine, Indianapolis, IN, USA.

Background: Activation of the Integrated Stress Response (ISR) by GCN2 is crucial for cells to survive in amino acid-depleted environments. We recently demonstrated in prostate cancer (PCa) that the GCN2 protein kinase that serves as a nutrient sensor in the ISR is critical for the regulation of transport of essential amino acids (EAAs) and for PCa growth and tumor progression. Inhibition of GCN2 results in lowered expression of amino acid transporters and a severe depletion of intracellular EAAs. Although loss of GCN2 in PCa cells reduces proliferation, this leads only in cell stasis and not cell death. We hypothesize that the senescent phenotype induced by GCN2 inhibition in PCa cells will render these cells vulnerable to cell cycle modulation and is a potential therapeutic target.
Methods: Immunoblot analyses was used to measure protein levels. Cell cycle analysis was performed by flow cytometry, and cell death/apoptosis was measured by Annexin-V and Cytotox-Red using an Incucyte.
Results: We determined that GCN2 inhibition in PCa cell lines results in G1-cell cycle arrest accompanied by induced p21 expression. p21 is a major regulator of the cell cycle by inhibition of cyclin/CDK complexes. Induced G1 arrest and p21 by GCN2 inhibition can be reversed by supplementation with EAAs, suggesting amino acid limitations is critical for the p21-dependent cell cycle checkpoint control. Combinations of GCN2 and p21 knockdowns using siRNAs resulted in cell death and apoptosis in PCa cells.
Conclusions: Our results suggest that inhibition of GCN2 in PCa cells causes severe starvation for EAAs, triggering the cell cycle checkpoint by a p21-dependent mechanism. Although PCa cells survive and undergo growth stasis following loss of GCN2, these cells are vulnerable to loss of p21 cell cycle control. We propose that GCN2 inhibition in combination with cell cycle modulators is novel therapy for the treatment of PCa.