Targeting the Stress Response Kinase GCN2 to Restore Immunity

in the Tumor Microenvironment

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Abstract

The tumor microenvironment (TME) is characterized by deficiencies in oxygen and key nutrients, such as glucose and amino acids, resulting in an overall immune-suppressive environment. Key suppressive cell types in the TME include tumor, stromal, and myeloid-derived suppressor cells (MDSC), which create a nutrient-poor environment that supports tumor growth and limits immune surveillance. Fludarabine (FLX) reduces tumor and MDSC activity by inhibiting GCN2 kinase. To further explore the utility of GCN2 as a therapeutic target, we tested the ability of two selective GCN2 inhibitors (GCN2i) (Buvana, FLX) to restore human CD8 T cell function through different mechanisms.

Results and Conclusions

- GCN2i selectively inhibited phosphorylation of GCN2 and EIF2a in human CD8+ T cells grown in amino-acid-depleted conditions.
- The inhibition of GCN2 increased human and mouse MDSC-mediated suppression and effector functions of CD8 T cells.
- GCN2i reversed mouse MDSC-mediated suppression more effectively than Argi and IDO.
- Treatment of human CD33+ MDSC alone with FLX-GCN2i reversed the suppressive function of MDSC on CD8 T cells.
- Thus, our data collectively demonstrates that GCN2 is a promising therapeutic target for the treatment of cancer.

FLX-GCN2i potently reduces EIF2a phosphorylation

FLX-GCN2i restores human T cell proliferation and function in glucose-starved conditions

FLX-GCN2i reverses human MDSC suppressive function and increases effector functions of human CD8+ T cells

FLX-GCN2i reverses suppressive function of mouse tumor-derived mMDSC and restores CD8 T cell effector functions

Assessment of GCN2 inhibition

- Biochemical
- Cellular

A) Potency and selectivity parameters for FLX-GCN2A: FLX-GCN2B and literature compound (I): Represents Nokama et al. at the inhibition of GCN2 kinases adrenaline (A) or cancer cells to express GCN2 through the use of enzymatic activity. Proc. Natl. Acad. Sci. USA. 2018;115(77):E7788. B) FLX-GCN2A and B were precipitated with recombinant human GCN2 and pef2a substrates. Enzymatic activity was measured by T-SREBP and used to demonstrate dose-dependent inhibition of pef2a. C) SKN-3 cells were precipitated with FLX-GCN2A/B for 2 hr and enzymatic activity was measured by AlamarBlue.