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Targeting the TCA Cycle with Histone Deacetylase and Nicotinamide Phosphoribosyltransferase Inhibitors Uncovers a Critical Role for YAP1 in Neuroendocrine Cells

Luigi Scotto PhD[1,2], Maryam Safari PhD[1,2], Ping Hou[2], Tito Fojo, MD PhD[1,2], Susan Bates MD[1,2].

[1]Columbia University, [2]James J. Peters VA Medical Center.

BACKGROUND

More than 12,000 people in the United States are diagnosed with a NET each year and approximately 175,000 people are living with this diagnosis. Little progress has been made in the therapy of NETs over the last two decades, and identification of new vulnerabilities remains a priority.

METHODS

We used two libraries of compounds selected for potential repurposing and identified agents with the highest cytotoxic activity in neuroendocrine models. FACS analysis was used to examine drug sensitivity; gene expression profiling was performed to gain insight into the molecular mechanisms responsible for drug sensitivity. Immunoblot and metabolic flux analysis were used to confirm observations at molecular and metabolic levels. Cell viability assays for synergy among drug-drug combinations were performed.

RESULTS

In the initial screen, nicotinamide phosphoribosyltransferase (NAMPT) and histone deacetylase (HDAC) inhibitors had the highest activity. Hits were validated in an expanded set of neuroendocrine cell lines and their mechanism of action examined. Differential sensitivity to NAMPT inhibitors was documented with gene expression profiles indicating up-regulation of genes involved in hypoxia, glycolysis, gluconeogenesis, and cholesterol homeostasis in NAMPT resistant cells, suggesting increased reliance on glucose and its conversion to pyruvate via glycolysis to meet energy requirements. Furthermore, metabolic flux analysis revealed that in sensitive cells, death following NAMPT inhibition results from a reduction in basal oxidative phosphorylation and energy production. Differential expression of YAP1, the yes-associated protein, between sensitive and resistant cells was indicative of a possible role in the observed drug resistance. Follow-up studies using Kelly cells ectopically expressing YAP1 (Kelly/YAP1), confirmed over-expression of YAP1 increases drug resistance, concurrent with an increase in glycolysis in metabolic flux analysis. Moreover, in resistant cells, interfering with YAP1 function or downregulating its expression increased NAMPT sensitivity, accompanied by a marked reduction in ATP production. Lastly, drug-drug combination using the HADC inhibitor romidepsin in combination with NAMPT inhibitors showed synergistic activity at low sub-lethal concentrations.

CONCLUSIONS

Exploiting metabolic vulnerabilities in neuroendocrine cells offers an opportunity for new therapeutic strategies. A double hit on the TCA cycle – depleting acetyl CoA via HDAC inhibition, previously shown, and blocking key intermediate steps dependent on NAD cofactors via NAMPT inhibition could be highly effective and should be pursued with a goal of developing translational clinical trials.

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