"Neuron-specific enolase" revisited: a new drug target in SDH-deficient paraganglioma?

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INTRODUCTION

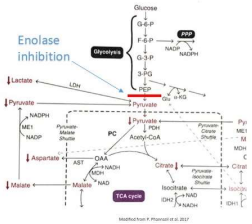
Pheochromocytomas (PC) and paragangliomas (PG) are rare, non-epithelial, often hereditary neuroendocrine tumors that arise respectively in the adrenal medulla or extra-adrenal paraganglia. A PC is an intra-adrenal PG. Loss-of-function mutations in genes encoding subunits of the TCA cycle enzyme succinate dehydrogenase (SDH) account for a large proportion of PC/PG. *SDHB* mutations are particularly associated with metastasis, and there is currently no cure for metastatic disease. A cell line and xenograft model of SDH-deficient PC called RS0 (for rat Sdhb-null) recently developed from rats with a heterozygous germline *Sdhb* mutation closely resembles *SDHB*-mutated human PCPG (1) and provides a tool for pre-clinical testing of new drugs targeting vulnerabilities conferred by SDH deficiency. A second, SDH-intact PC cell line called RS1/2 developed from the same rat lineage serves as a control:

RS0 (for Rat Sdh 0): Sdhb$^{-/-}$ lost the wild type allele

RS1/2: *Sdhb*$^{+/-}$ (from the same rat colony, lost the mutant allele by chromothripsis, Sdh functionally intact

RS1/2 serves as a control for studies of RS0 cells and tissues

Metabolic alterations in Sdh-deficient PC/PG include "rewiring" of the TCA cycle to replenish molecules needed for cell proliferation and increased utilization of glycolysis to produce enough energy for cell survival. This suggests enolase, the penultimate enzyme in the glycolysis pathway, as a drug target.



Enolase is a dimer combining subunits encoded by 3 separate genes ENO1, ENO2 or ENO 3, respectively encoding subunits α, γ or β. Enolase 2, also known as "Neuron-specific Enolase" (NSE) is a predominantly γγ dimer selectively expressed in neurons and neuroendocrine cells, recognized for decades as a marker for those cell types and corresponding tumors. A novel selective enolase 2 inhibitor called POMHEX has recently proven effective against ENO1 deficient human cancers in rodent xenografts (2). We hypothesized that POMHEX would selectively affect RS0 and not SDH-intact RS1/2 cells because of fragility caused by loss of Sdh.

- Enolase is a dimer encoded by 3 separate Genes
- Each isoenzyme is a typically homodimer composed of αα ββ, or γγ subunits,
- However, heterodimers are also known to exist

Type of enolase	Recognized name	Subunit	Distribution	Subunit dimers
Enolase 1	Nonneuronal enolase	ENO1 gene encodes α subunit	Adipose, brain (support cells), liver, spleen, and kidney*	αα, αγ, αβ
Enolase 2	Neuron-specific enolase	ENO2 gene encodes γ subunit	Neurons and neuroendocrine tissue, neuronal support cells*	γγ, αγ
Enolase 3	Muscle-specific enolase	ENO3 gene encodes β subunit	Muscle cells	ββ, αβ

S. Bezek, ... Viktoria Bogner-Flatz, in Biomarkers for Traumatic Brain Injury, 2020

Neurons switch from alpha enolase to gamma enolase (NSE) during development in rats and primates

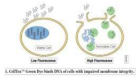
OBJECTIVES

1. Test for expression of NSE and Eno1 in RS0 cells, RS1/2 cells and normal rat adrenal
2. Test for selective cytotoxicity of POMHEX at concentrations feasible for treatment of PC/PG *in vivo*
3. Test the effects of POMHEX on glucose utilization and ATP synthesis to confirm on-target effects

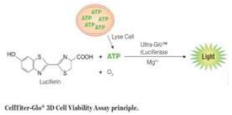
MATERIALS & METHODS

Expression of Eno1 and Eno2 was tested by immunohistochemistry and immunoblots using the same antibodies (Novus Biologicals NSP-25147, Polysciences NSE-Rat 17435A).

Cytotoxicity of POMHEX was tested in parallel against RS0 and RS1/2 cells by direct cell counts and a fluorimetric cell death assay (Cell Tox Green, Promega)



ATP levels before the onset of detectable cell death were measured by a quantitative luminescence assay (Cell Titer Glo, Promega) .

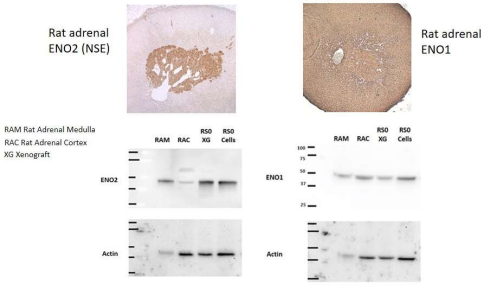


CellTiter-Glo® 3D Cell Viability Assay principle.

The of utilization of glucose was analyzed by NMR spectrometry tracing the metabolic fate of UC13-glucose

RESULTS

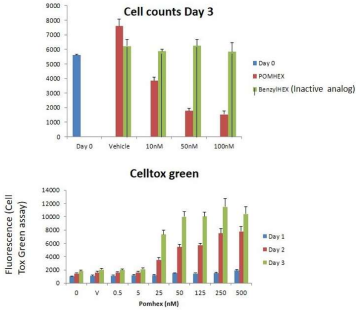
1. Although neurons switch from alpha to gamma enolase (NSE) during development, that does not seem to be fully the case in normal or neoplastic adrenal medulla, which coexpresses both isoforms



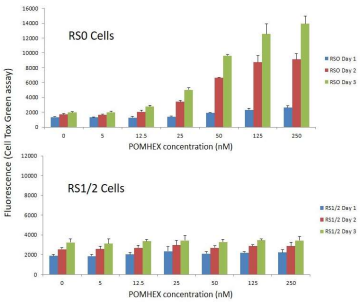
RESULTS (continued)

2. POMHEX is cytotoxic to RS0 cells but not RS1/2 cells at nanomolar concentrations compatible with in vivo use.

Effects of POMHEX on RS0 Cells

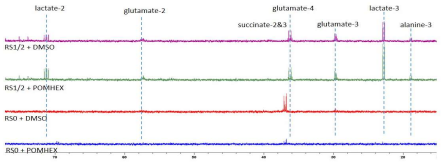


POMHEX Kills RS0 But Not RS1/2



3. Cell death is preceded by decreased incorporation of U-13C glucose into lactate, pyruvate and other metabolites and ATP depletion

POMHEX blocks utilization of 13C-U-glucose in RS0 but not RS1/2 cells

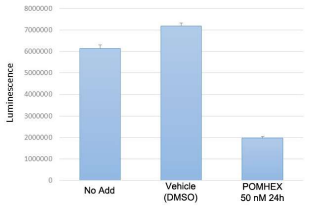


Cells were incubated in RPMI 1640 with 50nM POMHEX for 24h, with 2mg/mL 13C-U-glucose for the final 6h

Note that almost all label in control RS0 cells winds up in succinate

RESULTS (continued)

POMHEX depletes ATP in RS0 cells prior to the onset of cell death



CONCLUSIONS

Our findings in cell culture establish "NSE" as a new potential drug target and POMHEX as a prototype drug that could selectively target SDH-deficient tumors while minimizing bystander toxicity to SDH-intact cells

Determining why targeting of Eno2 is cytotoxic in cells that also express Eno1 may provide important insights into differential functions of these two isoforms.

The next steps are to test whether this proof of principle applies to in vivo xenografts. In addition, drug stability should be improved.

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