"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. SDH 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 SDH-mutated human PC/PG (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

Sdhb-Rat

Sdhb+/−

RS1/2

Sdhb+/+

RS0 and not SDH-intact RS1/2 cells because of fragility caused by loss of xenografts (2). We hypothesized that POMHEX would selectively affect proven effective against ENO1 deficient human cancers in rodent recognized for decades as a marker for those cell types and corresponding ENO1, ENO2 or ENO3, respectively encoding subunits α, γ or β. Enolase Enolase is a dimer combining subunits encoded by 3 separate genes "Neuron-specific enolase" (NSE) is a predominantly γγ isoform of enolase, the penultimate enzyme in the glycolysis pathway, as a drug target.

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

RESULTS (continued)

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).

The of utilization of glucose was analyzed by NMR spectrometry tracing the metabolic fate of U-13C-glucose

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

REFERENCES


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