

## MITOCHONDRIAL 2HG PRODUCTION AS A FUNCTION OF IDH2 AND HOT IN BREAST CANCER CELLS

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Cancer metabolic alterations result from complex genetic and epigenetic adjustments, and include also mitochondrial pathways glutaminolysis, reductive carboxylation (RC), oncometabolite 2-hydroxyglutarate (2HG) production, and NADPH synthesis. We studied complex mechanisms that promote mitochondrial enzymes isocitrate dehydrogenase 2 (IDH2) and hydroxyacid-oxoacid transhydrogenase (HOT) towards 2HG production in breast cancer cell lines. We demonstrate that IDH2 enzyme produces 2HG *in vitro*, as assumed from functional analysis of isolated recombinant wild-type IDH2. Our analysis of metabolic flux shows that mitochondrial production of 2HG by wild-type IDH2 is largely dependent on mitochondrial NADPH balance, because induction of mitochondrial NADPH by dm-L-malate or overexpression of NADPH-producing enzymes induce IDH2-dependent 2HG synthesis. In addition, we demonstrate that active interplay and competition between IDH2 and HOT for substrate (2OG) exists; overexpression of superactive mutant of glutaminase 1, which induces 2OG production, favors HOT when NADPH levels are low. Moreover, we demonstrate that IDH2 is a direct substrate of mitochondrial deacetylase SIRT3, and that distinct regulation by SIRT3 towards oxidative vs. reductive IDH2 activity exists. Quantity and frequency of acetylated lysines on IDH2 declines when treated with SIRT3. The metabolic flux analysis shows that mitochondrial production of 2HG by wild-type IDH2 depends on SIRT3 presence and activity in mitochondria, as supposed by overexpression of wild-type SIRT3 and SIRT3-inactive mutant in cancer cells. Moreover, an acetylation surrogate IDH2 mutant K413Q tends to decrease levels of 2HG *in vitro*. Taken together, our findings impact the understanding of breast cancer metabolism, since breast cancer express a broad range of IDH2, HOT and SIRT3 levels, and exhibit distinct metabolic phenotypes, including 2HG levels. *Supported by The Czech Science Foundation grant 16-04788S to P.J.*