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Knockdown of both mitochondrial isocitrate dehydrogenase enzymes in pancreatic beta cells inhibits insulin secretion

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ABSTRACT

Background: There are three isocitrate dehydrogenases (IDHs) in the pancreatic insulin cell; IDH1 (cytosolic) and IDH2 (mitochondrial) use NADP(H). IDH3 is mitochondrial, uses NAD(H) and was believed to be the IDH that supports the citric acid cycle.
Methods: With shRNAs targeting mRNAs for these enzymes we generated cell lines from INS-1 832/13 cells with severe (80%–90%) knockdown of the mitochondrial IDHs separately and together in the same cell line.
Results: With knockdown of both mitochondrial IDH's mRNA, enzyme activity and protein level, (but not with knockdown of only one mitochondrial IDH) glucose- and BCH (an allosteric activator of glutamate dehydrogenase)-plus-glutamine-stimulated insulin release were inhibited. Cellular levels of citrate, α-ketoglutarate, malate and ATP were altered in patterns consistent with blockage at the mitochondrial IDH reactions. We were able to generate only 50% knockdown of Idh1 mRNA in multiple cell lines (without inhibition of insulin release) possibly because greater knockdown of IDH1 was not compatible with cell line survival.
Conclusions: The mitochondrial IDHs are redundant for insulin secretion. When both enzymes are severely knocked down, their low activities (possibly assisted by transport of IDH products and other metabolic intermediates from the cytosol into mitochondria) are sufficient for cell growth, but inadequate for insulin secretion when the requirement for intermediates is certainly more rapid. The results also indicate that IDH2 can support the citric acid cycle.
General significance: As almost all mammalian cells possess substantial amounts of all three IDH enzymes, the biological principles suggested by these results are probably extrapolatable to many tissues.

1. Introduction

The original purpose of the work reported here was to explore the possible redundancy of function among the three isocitrate dehydrogenase (IDH) enzymes in insulin secretion by using shRNA to generate beta cell lines with stable knockdown of each of the three IDH isoforms. There are three mammalian IDHs; two mitochondrial enzymes and one cytosolic enzyme in most mammalian cells. These enzymes catalyze the reversible reaction: NAD(P) + isocitrate ↔ NAD(P)H + α-ketoglutarate. The cytosolic enzyme (IDH1)[1] is an NADP-dependent enzyme that is highly homologous (70%) to the mitochondrial NADP-IDH (IDH2). These two enzymes are homo-dimers. The other mitochondrial IDH, IDH3, is a multimeric NAD-dependent enzyme encoded by three separate genes: IDH3a, IDH3b, and IDH3c. This enzyme is not homologous with either of the NADP IDHs in any of its subunits. The three subunits appear to share substrate binding and enzyme activity, but cannot substitute for each other.

Recent evidence from non-beta cells indicates that there could be redundancy in function among the IDH enzymes. The IDH3 enzyme was thought to be an essential element of the citric acid cycle, catalyzing the oxidation of isocitrate to α-ketoglutarate with the reduction of NAD to NADH. However, individuals have been reported who have homozygous mutations of the IDH3c subunit, resulting in essentially complete loss of IDH3 enzyme activity. Surprisingly, loss of this enzyme is apparently detrimental only in the eye, as the only reported finding is retinitis pigmentosa [1]. This suggests that the two mitochondrial enzymes could be substantially redundant in function. It is also possible that the cytosolic IDH enzyme (IDH1) reaction might compensate for loss of the mitochondrial IDH2 or IDH3 by producing metabolites in the cytosol that can be transported into mitochondria, as there is rapid transport of citrate, isocitrate, α-ketoglutarate and other metabolites between the cytosol and mitochondria. Any precursor, substrate or product of the IDH reaction, can be (re)imported into the mitochondrion and used in the citric acid cycle and for other mitochondrial pathways (Fig. 1).

In addition to a possible redundancy among the three IDH reactions, there also exists the possibility of redundancy of function between the

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[1] Abbreviations: BCH, 2-aminobicyclo [2,2,1]heptane-2-carboxylic acid; IDH1, cytosolic NADP isocitrate dehydrogenase; IDH2, mitochondrial NADP isocitrate dehydrogenase; IDH3, mitochondrial NAD isocitrate dehydrogenase; shRNA, short hairpin RNA; siRNA, small interfering RNA;

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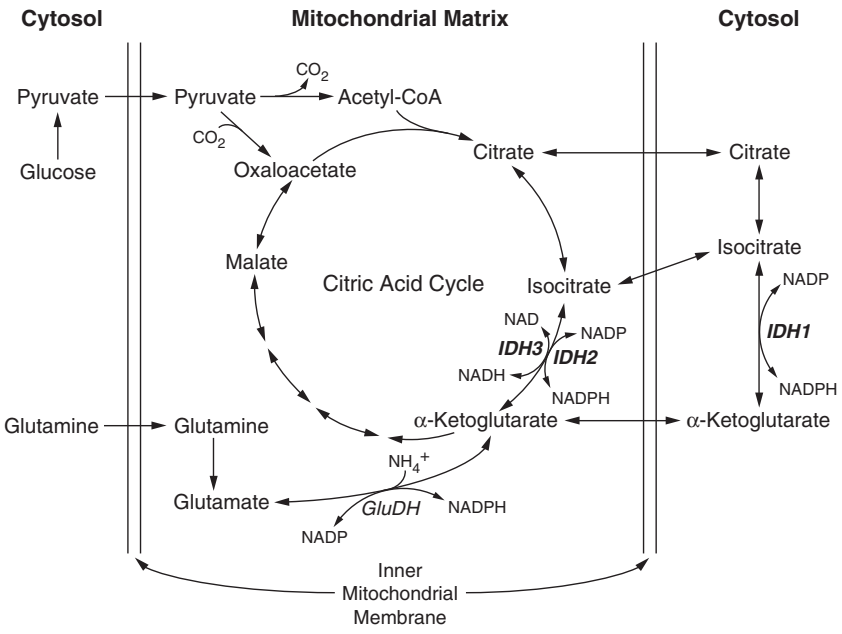

Fig. 1. Pathways of precursors for substrates and products of the three isocitrate dehydrogenase reactions in the pancreatic beta cell. Abbreviations: *GluDH*, glutamate dehydrogenase; *IDH*, isocitrate dehydrogenase.

IDHs and non-IDH enzymes. For example, the movement of citric acid cycle intermediates between the mitochondria and the cytosol has been proposed to participate in reactions that produce NADPH in the cytosol and play a role in insulin secretion by the pancreatic beta cell [2–6]. These reactions occur in cycles or shuttles in which the exported metabolite is oxidized by NADP to produce NADPH in the cytosol and the oxidized metabolite is reimported into the mitochondria for reduction and re-exportation to the cytosol. These shuttles of oxidized and reduced metabolites carry pyridine nucleotide NAD(H) and NADP(H) equivalents across the inner mitochondrial membrane and have evolved because pyridine nucleotides cannot penetrate the inner mitochondrial membrane. An isocitrate shuttle involving either or both of the mitochondrial IDHs and the cytosolic IDH has been proposed as one NADPH shuttle [3,6]. Previously NADPH shuttles involving mitochondrially exported malate and the cytosolic malic enzyme (the pyruvate malate shuttle) [2,3] and citrate exported to the cytosol involving the cytosolic enzymes ATP citrate lyase, cytosolic malate dehydrogenase and the cytosolic malic enzyme (the classic citrate pyruvate shuttle) were proposed to exist in the beta cell and there is fairly good evidence for each of these [2–5].

With one exception there is no report of knockdown of any of the IDH isoforms in the beta cell. One group reported that the knockdown of the cytosolic IDH (IDH1) mRNA 78% with siRNA, but with only 39% knockdown of IDH1 enzyme activity, in the INS-1 832/13 cell line, inhibited glucose-stimulated insulin secretion 59% [6]. In view of the redundancy of the IDH1 reaction in the cytosol with the IDH2 and IDH3 reactions in the mitochondria that each can catalyze the interconversion of α-ketoglutarate and isocitrate (Fig. 1), as well as other cytosolic enzymes that can participate in a NADP(H) shuttle (the cytosolic malic enzyme) [2–5,7], it is puzzling why knockdown of a single IDH enzyme should impair insulin secretion unless it plays a unique and nonredundant role in insulin secretion.

To further explore the possible redundancy of IDH enzymes in insulin secretion we attempted to generate INS-1 832/13-derived cell lines with knockdown of all three IDH isoforms and with knockdown of more than one of the IDHs in a single cell line. We were only partially successful even though we used a very efficient method of gene targeting (Tol2). We generated separate cell lines with strong stable knockdown of the individual mRNAs, proteins and enzyme activities of each of the mitochondrial IDHs, but glucose-stimulated insulin release was not inhibited in these cell lines. However, when we generated a cell line with severe knockdown of both mitochondrial isocitrate dehydrogenases together, we did observe inhibition of glucose- and BCH-plus glutamine-stimulated insulin release. We were unable to generate viable beta cell lines with more than 50% knockdown of the cytosolic NADP IDH (Idh1) mRNA. IDH1 enzyme activity was normal and insulin release was not inhibited in these cell lines. In addition, in an attempt to knockdown both NADP IDHs in a single cell line, the mitochondrial NADP-IDH enzyme (IDH2) was severely knocked down, but the cytosolic NADP-IDH (Idh1) mRNA was not knocked down. We also attempted to knock down the cytosolic malic enzyme and the cytosolic IDH in the same cell line. Malic enzyme activity was severely knocked down, but the cytosolic IDH enzyme activity was normal. We concluded that the severe knockdown of the cytosolic NADP IDH might not have permitted the development of viable cell lines. Herein we report a study of these cell lines.

2. Material and methods

2.1. Construction of targeted clones

shRNA vectors were constructed in the vector pSilencer 2.1U6 Hygro + Tol2 [7,8]. All insert sequences were confirmed by sequencing, and the resulting plasmids were transfected into the cell line INS-1 832/13 using Fugene HD (Roche) according to product directions. Cell populations were selected at least 3–4 weeks with Hygromycin (150 μg/ml, Invivogen) prior to testing, as described previously [7]. For double knockouts, pSilencer 2.1-U6 Puro (Applied Biosystems) was similarly modified to contain the Tol2 transposition site, and was used to create stable transferent populations with reduced Idh2 levels. Cells were first selected for at least 3–4 weeks with puromycin (200 ng/ml, Invivogen), and were subsequently transfected with the pSilencer 2.1-U6 Hygro + Tol2 plasmids targeting other genes and selected for at least 3–4 weeks. Selection was maintained until testing. All transfections included an equimolar amount of the pCMV-Tol2 vector, which encodes the Tol2 transposase, to increase efficiency of transfection [7,8]. Target sequences are shown in Supplementary Table 1 online.