The role of γ2 AMPK in islet secretory physiology

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Background
Impaired islet β-cell function is central to the pathophysiology of type 2 diabetes mellitus (T2D). Genome-wide association studies have implicated a number of predisposing genetic variants, the majority of which appear to alter β-cell function, mass or development. As a corollary, islets from donors with T2D exhibit a 50% reduction in glucose-stimulated insulin secretion (GSIS) compared with non-diabetic donors. These findings, reinforced by the success of multiple agents influencing β-cell insulin secretion (e.g. sulphonylureas, thiazolidinediones and incretin-based therapeutics) highlight the translational promise of targeting β-cell secretory function. An attractive therapeutic candidate is AMP-activated protein kinase (AMPK), a phylogenetically conserved multisubstrate kinase sensor of energetic stress which maintains energy homeostasis at cellular and whole-organismal level. AMPK activity is strongly regulated by physiologic changes in glucose in both islet β and α-cells. Adenoviral overexpression of tonically active AMPK blocks insulin secretion in clonal β-cells, rodent and human islets, and induces β-cell apoptosis in vivo. In murine clonal α-cells, adenoviral-mediated increases in AMPK activity stimulate glucagon release. AMPK’s regulation of β-cell secretory function appears pleiotropic, involving direct interactions with proteins integral to insulin exocytosis, β-cell differentiation and cell-cell interaction, transcriptional effects and likely altered metabolic intermediates. AMPK exists as a heterotrimer composed of a catalytic α subunit and regulatory β and γ subunits. While the effects of in vivo deletion of AMPK α subunits have been investigated, the specific contribution of γ2 to islet endocrine secretion is unknown.

Hypothesis
γ2 AMPK is a physiological regulator of glucose-induced insulin secretion from pancreatic islet β-cells and of glucagon secretion from α-cells that can be targeted to treat T2D.

We aim to test whether
i) γ2 AMPK is a physiological negative regulator of β-cell insulin secretion;
ii) γ2 AMPK positively regulates α-cell glucagon secretion;
iii) Modulating γ2 AMPK-specific function/downstream signalling can enhance normal β-cell insulin secretory capacity and inhibit α-cell glucagon release in vitro and applied to in vivo models of T2D.

Aims and description of the work
By integrating insights from highly specific murine and human in vitro β-cell models, this Fellowship will seek to define a specific role for γ2 AMPK in islet endocrine secretory physiology and determine its potential utility as a novel pharmacological target for T2D.
1. Define normal islet expression of the γ regulatory subunit isoforms (γ1/γ2) at transcript (ISH)/protein (IHC) level in human and murine islets, and the human β-cell line EndoC-BH2.
2. Characterise the functional consequences of γ2 loss-of-function on glucose homeostasis and islet secretory capacity in vivo. Highly-restricted γ2 AMPK deletion in islet β-cells and α-cells will be
achieved by crossing Prkag2<sup>loxP/loxP</sup> mice with mice expressing Cre recombinase under the control of the Ins1 and glucagon promoters (Glu-cre), respectively, followed by assessment of: glucose and insulin tolerance, islet cytoarchitecture, β/α-cell ultrastructure, isolated islet insulin and glucagon secretion, islet glucose metabolism (glucose-induced change in [Ca<sup>2+</sup>]), glucose sensing (β-cell whole-cell K<sub>a</sub><sub>ATP</sub> conductance) and hormone exocytosis (via membrane capacitance).

3. Evaluate the role of γ2 function in the human β-cell line (EndoC-βH2) using siRNA-based loss-of-function studies. Readouts: insulin secretion/content, gene expression, secretory dynamics.

4. Explore signalling mechanisms linking γ2 AMPK to altered islet endocrine secretion with subsequent targeting to potentiate GSIS. Hypothesis-driven assessment will include evaluation of ghrelin signalling which is insulinostatic via UCP2 and IA-2β, and affects glucagon secretion.

Non-hypothesis based study of regulated gene networks (RNASeq) and impact on downstream AMPK targets (directed phosphoproteome and metabolome) in γ2 β-cell KO islets and γ2 siRNA- transfected EndoC-βH2 cells. Insights gained will be utilised to target key nodes in the signalling pathways in vitro and, if successful, translated to in vivo murine models of T2D.

**Significance/justification**

In completing the work outlined, the Fellow will be enabled to implicate a specific and novel role for γ2 AMPK in physiological islet insulin and glucagon secretion, begin to delineate the underlying signal transduction mechanisms involved and assess the utility of modulating γ2 AMPK signalling to simultaneously target the bihormonal endocrinopathy characteristic of the T2D pancreatic islet.

**References**

Recent relevant publications


