



Leveraging the cell cytoskeleton to produce GLP1R agonists with improved efficacy for beta cell preservation, nonalcoholic steatohepatitis resolution and gene therapy

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Project outline

Background: Glucagon-like peptide-1 receptor (GLP1R) agonists (GLPRA) have become a mainstay of type 2 diabetes treatment due to their effects on insulin secretion and food intake¹. However, GLP1RA cannot reverse the decline in beta cell mass, only alter disease trajectory, meaning that many patients eventually need treatment escalation. GLP1RA are also effective in a number of other metabolic diseases including NASH. However, we and others have shown that GLP1R is only present in CD8+ T-cell and endothelial cell subpopulations^{2,3}, which could partly explain the modest effects of GLP1RA on NASH resolution (23% versus placebo). Lastly, our recent studies have shown the utility of GLP1R as a beta cell-specific molecular address for cargo delivery^{4,5}. However, translation to human beta cells and iPSC-derived beta-like cells is complicated by differences in GLP1R expression/trafficking, impeding target discovery and RNAi therapy. Going forwards, it is conceivable that similar approaches could target liver-resident GLP1R+ CD8+ T-cells and endothelial cells for RNAi/immunotherapy.

Objective: We propose to develop a GLP1RA conjugate, which: 1) promotes a differentiated beta cell status through increased GLP1R expression and cytoskeletal modification; 2) access and bind immune/stromal cell compartments involved in liver inflammation/fibrosis; and 3) enhance GLP1R expression and trafficking for cell-specific delivery of RNAi and other gene therapy.

Approach The GLP1R-conjugate will be based upon a novel islet-specific actin-severing protein (ASP) that we recently discovered. Deletion of this ASP leads to changes in beta-cell function. Most recently, we have begun to investigate the role of this ASP in GLP1R signaling and beta cell function, since cytoskeletal rearrangement is critical for GPCR function⁶, hormone secretion⁷ as well as beta cell maturity⁸.

Hypothesis: Cell cytoskeleton modulation increases GLP1RA efficacy by influencing GLP1R expression, cell differentiation and access to low abundance targets.

Description of work:

Aim 1: Synthesize chimeric exendin4-ASP(Ex4-ASP), before characterization of GLP1R signaling potency/bias with cAMP, beta-arrestin, Ca²⁺ and kinetic assays. Using human islets and iPSC-derived

islet-like structures, the effects of Ex4-ASP on beta cell differentiation (PDX1, NKX6-1, MAFA/MAFB), insulin secretion, and GLP1R expression will be examined.

Aim 2: Effects of Ex4-ASP on NASH progression will be examined in choline-deficient mouse models. Fluorescent Ex4-ASP congeners will be examined for access and binding to liver-resident immune (CD8+) and stromal cells (endothelium) using human liver samples taken from healthy and obese/NAFLD/NASH donors.

Aim 3: Ex4-ASP-stimulated GLP1R trafficking will be examined in human islets and iPSC-derived islet-like structures using Novo Nordisk Mab 3F52 antibody. Single molecule imaging will quantify Ex4-ASP-stimulated GLP1R membrane nanodomains, as well as interactions with the F-actin cytoskeleton.

Aim 4: Immunomodulatory ASP conjugates are in phase 2 trials. We will thus use structure-activity-relationships (SARs) to identify novel ASP forms lacking binding of other cell components. Novel ASP forms will then be chemically conjugated to the second generation GLP1RA semaglutide.

Approaches: 1) DiscoverX assays in SNAP_GLP1R CHO cells; 2) differentiation screens in iPSC and human islets; 3) steatosis scoring in choline-deficient mice; 4) FACS for Ex4-ASP-labelled CD8+ T-cells and endothelial cells (CD45-); 4) confocal imaging using Olympus FV3000 (NNRCO); and 5) single-molecule imaging using Abberior dSTORM (OCDEM).

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2. [Super-resolution microscopy compatible fluorescent probes reveal endogenous glucagon-like peptide-1 receptor distribution and dynamics.](#) Ast J, Arvaniti A, Fine NHF, Nasteska D, Ashford FB, Stamatakis Z, Koszegi Z, Bacon A, Jones BJ, Lucey MA, Sasaki S, Brierley DI, Hastoy B, Tomas A, D'Agostino G, Reimann F, Lynn FC, Reissaus CA, Linnemann AK, D'Este E, Calebiro D, Trapp

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3. [Conditional and Reversible Activation of Class A and B G Protein-Coupled Receptors Using Tethered Pharmacology](#). Podewin T, Ast J, Broichhagen J, Fine NHF, Nasteska D, Leippe P, Gailer M, Buenaventura T, Kanda N, Jones BJ, M'Kadmi C, Baneres JL, Marie J, Tomas A, Trauner D, Hoffmann-Röder A, **Hodson DJ**. ACS Cent Sci. 2018 Feb 28;4(2):166-179. doi: 10.1021/acscentsci.7b00237. Epub 2018 Jan 16.PMID: 29532016
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