Endoc-BH5, Primary-like Human Beta Cell Model: Finally!! A Robust and Reliable Cell Line for T1D and T2D Disease Modelling

Incretin pharmacology, T lymphocyte beta cell killing, cytokine inflammation and Glucolipotoxicity for drug screening and target validation

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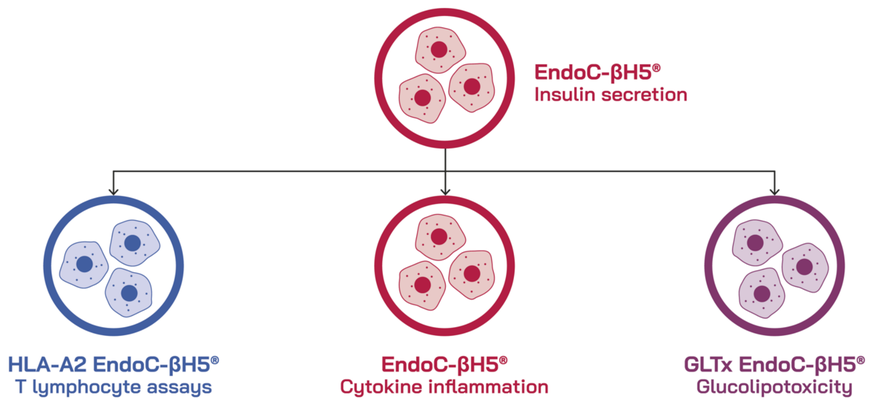
BACKGROUND AND OBJECTIVES

Due to the limitations of current options, diabetes research and drug discovery would strongly benefit from reproducible and easily accessible human beta cells. Our aim was to create a human primary-like beta cell line that can be used to address all in vitro disease modelling needs.

In this study, we developed the unique EndoC-βH5 through integrative gene transfer of immortalizing transgenes into the human fetal pancreas. Subsequently, EndoC-βH5 was utilized to develop other models and protocols for glucolipotoxicity, cytokine-mediated inflammation, T lymphocyte activation, and mediated killing. With their robust and strong insulin secretion, EndoC-βH5 cells can be employed in insulin secretagogue screening assays. This was demonstrated by their reproducible response to glucose, GLP1, GIP, and glucagon. Furthermore, we created a derivative, GLTx EndoC-βH5, which models glucolipotoxicity. GLTx EndoC-βH5 undergoes cytotoxicity when cultured in the presence of palmitate and elevated glucose, thus mimicking type 2 diabetes. We also developed a model of cytokine-mediated inflammation in EndoC-βH5 cells. Cytokine-treated EndoC-βH5 cells undergo cell death accompanied by impaired insulin secretion, confirming the mechanistic relevance of the model. Importantly, these effects are prevented by the JAK1/2 inhibitor, Baricitinib, which is approved for rheumatoid arthritis treatment. Finally, we addressed the modelling of type 1 diabetes with beta cell/T lymphocyte interaction assays. We generated HLA-A2-expressing EndoC-βH5 cells, which elicit HLA-A2 allo-reactive CD8 T cell activation. This effect is potentiated by IFNγ and blocked by Baricitinib. Additionally, we demonstrated HLA-A2 EndoC-βH5-specific killing by diabetogenic HLA-A2-restricted prepro-insulin PPI15-24 epitope-recognizing CD8 T lymphocytes.

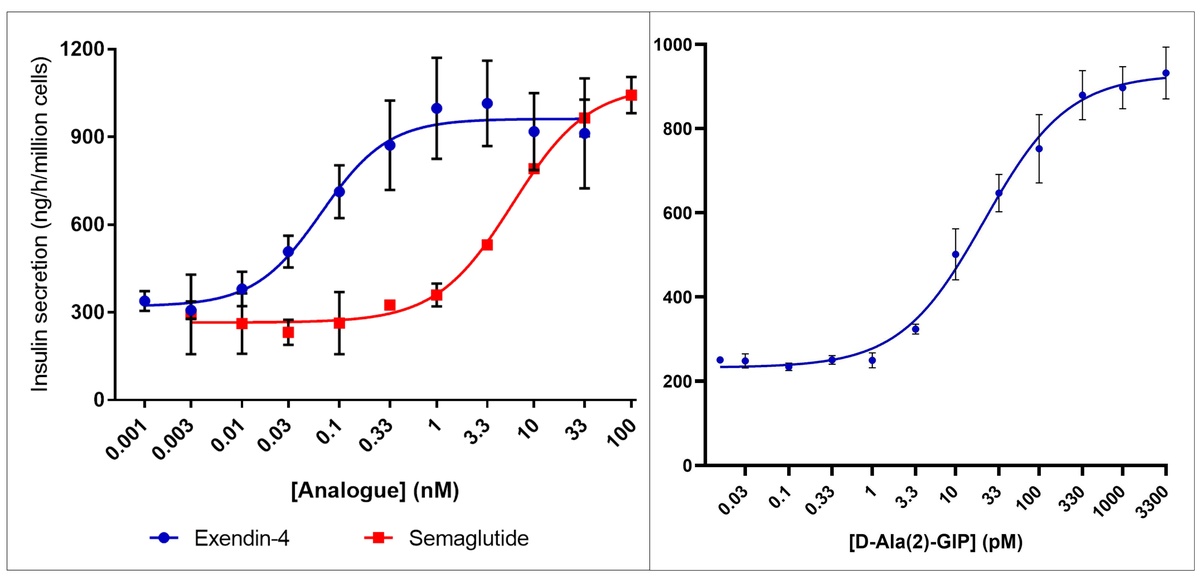
Overall, EndoC-βH5 cells and their derivatives allowed us to model several beta cell defects associated with T1D and T2D. As a result, they represent valuable models for screening new therapeutic approaches, validating candidate molecules, and characterizing molecular targets at small, medium, and large scales, taking advantage of readily available and highly reproducible EndoC-βH5 cells.

EndoC-βH5®: from 100% functional healthy human beta cells to a unique panel of physiological type 1 and type 2 diabetes models



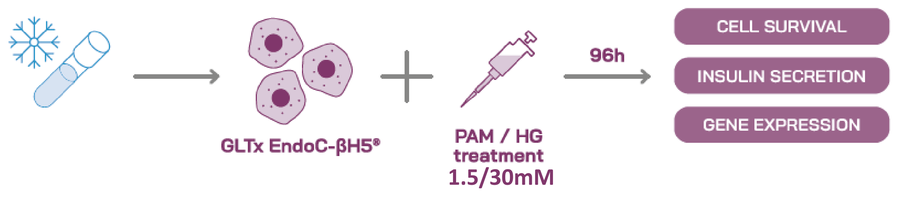
Ready to use human beta cells for rapid disease modeling and results

Dose dependant responses to GLP-1 and GIP analogues

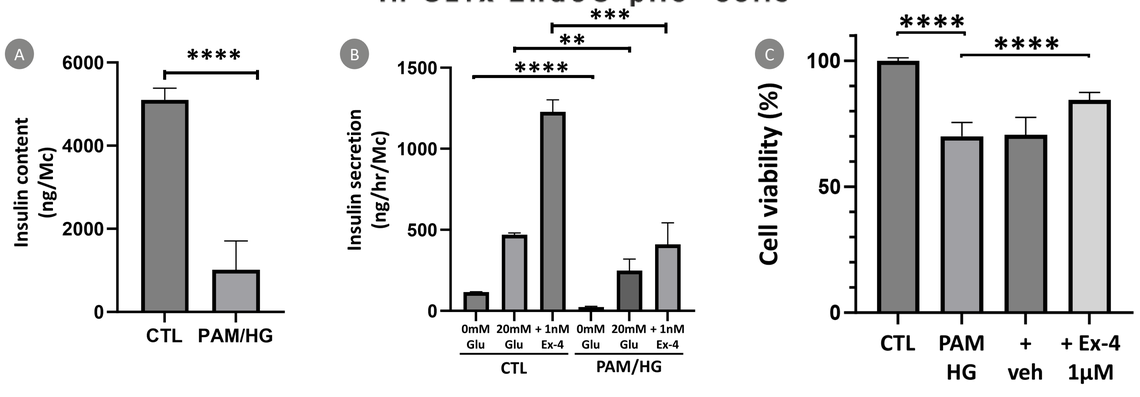


EndoC-βH5® dose dependently respond to GLP-1 and GIP receptor analogues Exendin-4, Semaglutide and D-Ala(2)-GIP. GSIS assay results showing Exendin-4, Semaglutide and D-Ala(2)-GIP responses in presence of 11mM Glucose. For all three agonists, potentiation of insulin secretion is up to 3.5-fold compared to 11mM glucose stimulation.

GLUCOLIPOTOXICITY



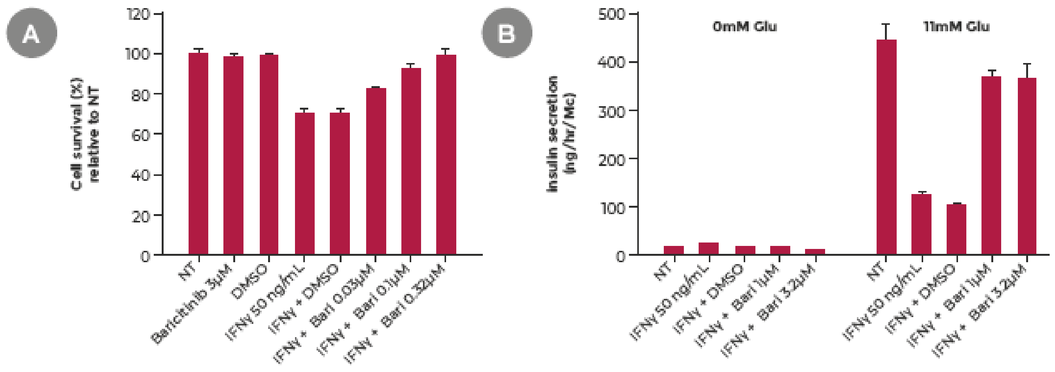
Palmitate/glucose treatment induces cytotoxicity in GLTx EndoC-βH5® cells



Palmitate/glucose treatment induces glucolipotoxicity and beta cell death in GLTx EndoC- βH5® cells relative to non treated GLTx EndoC-βH5®. GLTx EndoC-βH5® cells were treated with 30mM glucose and 1.5mM palmitate for 96 hrs. n=4.
A and B High concentrations of Palmitate and glucose significantly caused a beta cell dysfunction by decreasing insulin content and responsiveness to glucose and incretins.
C Palmitate and glucose induced cytotoxicity in GLTx EndoC-βH5® is partially prevented by 1µM GLP-1R signalling

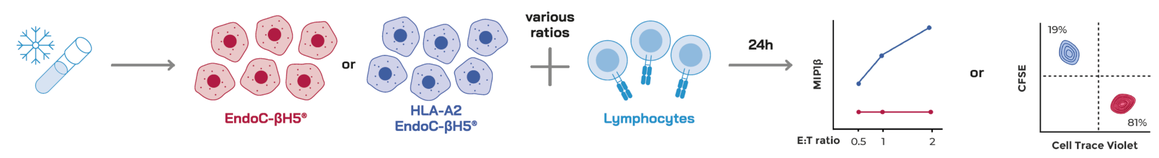
CYTOKINE MEDIATED INFLAMMATION

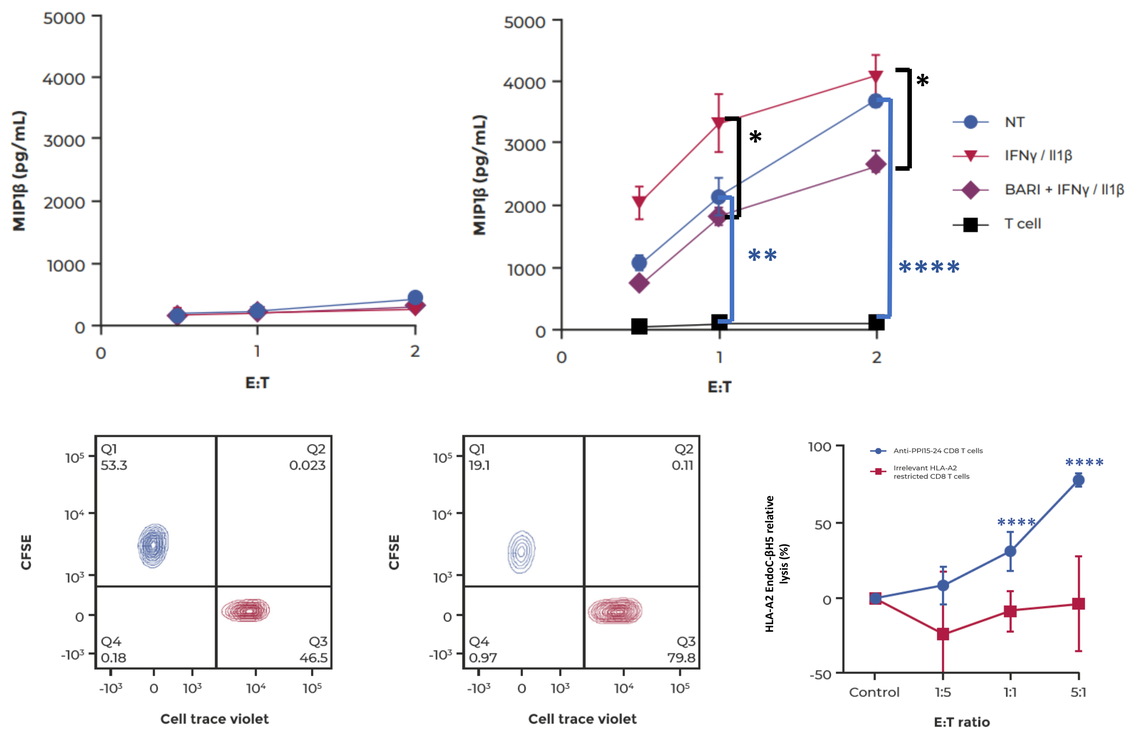
Cytokine treatment induces cytotoxicity and impaired insulin secretion in EndoC-βH5® cells



IFNγ treatment in EndoC-βH5® cells induces beta cell death and impaired insulin secretion :
A) Histogram showing cell survival relative to non treated cells in EndoC-βH5® cells treated with IFNγ (50 ng/mL, 96 hrs) or IFNγ + Baricitinib (Bari). IFNγ induces beta cell death which is dose dependently prevented by Baricitinib, n=3.
B) Representative histogram showing basal (0mM glucose) and stimulated (11mM glucose) insulin secretion, normalized to cell number, in EndoC-βH5® cells treated with IFNγ or IFNγ + Baricitinib. IFNγ impairs insulin secretion which is partially normalized by Baricitinib.

CD8 T CELL ACTIVATION AND T CELL MEDIATED BETA CELL KILLING



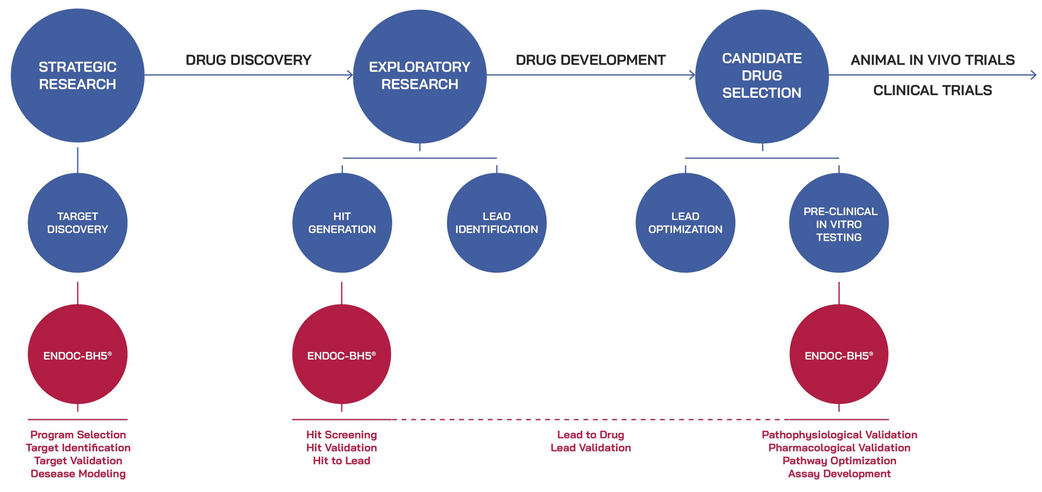


HLA-A2 EndoC-βH5® cells activate CD8 T lymphocytes:
MIP1β secretion by CD8 T cells that were co-cultured for 24h with HLA-A2 EndoC-βH5® cells but not control EndoC-βH5® cells, as a marker of CD8 T cell activation. T cell activation is further potentiated by treating HLA-A2 EndoC-βH5® cells with IFNγ (1000 U/ml) and Il1β (2 ng/ml) and partially prevented by JAK1/2 inhibitor Baricitinib (BARI) (4µM), n=3.
HLA-A2 EndoC-βH5® cells are specifically killed by HLA-A2 restricted anti-PPI15-24 CD8 T lymphocytes:
Representative flow cytometry results showing relative number of live control (Cell trace violet, red) and HLA-A2 (CFSE, blue) EndoC-βH5® cells after their coculture in absence or presence of HLA-A2 restricted anti-PPI15-24 CD8 T lymphocytes (E:T ratio 5:1). Specific killing of HLA-A2 EndoC-βH5® cells (blue line) by HLA-A2 restricted anti-PPI15-24 CD8 T lymphocytes but not irrelevant HLA-A2 restricted T cells, n=3.

CONCLUSION

Take advantage of EndoC-βH5® unique properties to study type 1 and type 2 diabetes using newly developed disease relevant models and functional assays:

- √ 100% pure population of functional human beta cells
- √ Reproducible data generation between batches
- √ Readily available as frozen systems
- √ GLP-1/GIP signaling



PHYSIOLOGICAL READOUTS

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