From GLP-1 to Amylin: Development of Reporter Cell Lines for Next-Generation Metabolic Therapeutics

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BACKGROUND

The global rise in obesity has led to increased cases of type 2 diabetes (T2D), cardiovascular diseases, and certain cancers, making weight control and management of related comorbidities a priority. Incretin agonists such as GPL-1 improve glucose control, promote satiety, support weight loss, and reduce cardiovascular risk. Dual or triple receptor agonists targeting GLP-1R, GCGR (glucagon receptor) and GIPR (glucose-dependent insulinotropic polypeptide receptor) show even greater promise for metabolic and weight management.

Amylin and Leptin receptors also regulate energy balance, appetite, metabolism, and glucose homeostasis. Agonists targeting these receptors, alone or in combination with GLP-1, are under development to enhance therapeutic outcomes by offering improved glycemic control and deeper weight loss. However, despite the remarkable progress achieved over the last decade, these agonists also cause substantial muscle loss. To address this, researchers are exploring Activin/Myostatin pathway inhibitors as a strategy to preserve muscle while promoting weight loss.

To support the discovery and assessment of drug candidates, we developed a series of luciferase reporter cell lines measuring GLP-1R, GLP-2R, GIPR, GCGR, Activin/myostatin receptor, Leptin receptor, and Amylin receptor responses.

CELL LINES AND CHARACTERIZATION

Cell Lines

Single clone, stable HEK293 (Human Embryonic Kidney 293) cell lines were engineered to conditionally express firefly luciferase under the control of the appropriate response elements (Table 1). They also overexpress one of the incretin receptors where relevant.

Chemicals

Chemicals used in this study were purchased from MedChemExpress, R&D Systems, and GenScript. GLP-1, Fc-Fusion Recombinant was produced at BPS Bioscience (#102006).

Methods

Experiments were performed in triplicate in white, clear-bottom cell culture plates. Cells were seeded in 90 µl/well of Opti-MEM™ medium (ThermoFisher Scientific) and allowed to attach overnight. The next day, serial dilutions of agonists were prepared in Opti-MEM™ and added to the cells (10 µl/well). Medium without agonist was added to the unstimulated control wells.

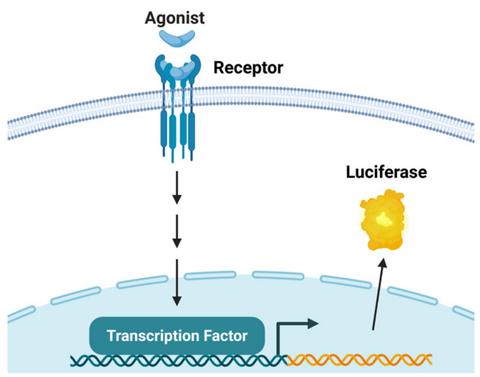

Figure 1. Illustration of the general mechanism of reporter activation in a luciferase reporter cell line.

The cells were then incubated at 37°C with 5% CO_2 for 5 hours. After incubation, 100 µl/well of ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was added to each well at 100 µl/well and rocked for 15 minutes at room temperature. Luminescence was measured using the BioTek Synergy 2 plate reader. The background luminescence was determined against a no-cell condition and was subtracted from all other values.

Cell Line	Catalog #	Response Element	Overexpression
GLP-1R/CRE Luciferase Reporter	78176	cAMP response elements (CRE)	GLP-1R
GLP-2R/CRE Luciferase Reporter	83623	cAMP response elements (CRE)	GLP-2R
GIPR/CRE Luciferase Reporter	78589	cAMP response elements (CRE)	GIPR
Glucagon Receptor (GCGR)/CRE Luciferase Reporter	82187	cAMP response elements (CRE)	GCGR
TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter	60653	SMAD binding elements (SBE)	n/a
Leptin Responsive Luciferase Reporter	83622	STAT3 response elements	Leptin R
Amylin Receptor 3 (AMY3R)/CRE Luciferase Reporter	83544	cAMP response elements (CRE)	CALCR and RAMP3

Table 1. Characteristics of the HEK293 luciferase reporter cell lines described in this study.

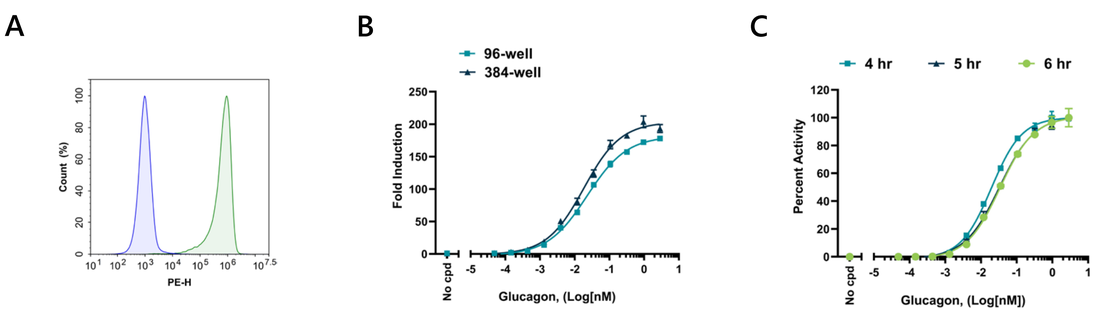

Figure 2. Example of cell line characterization. Cell surface expression of Glucagon receptor in GCGR luciferase cell line (A)*. Receptor expression was evaluated by flow cytometry using a specific primary antibody (R&D Systems, MAB10296-SP), and secondary PE-conjugated anti-mouse IgG (BioLegend Poly4053). Overexpressing cells (green) were compared to parental luciferase reporter HEK293 cells (blue).* ***Agonist-induced luciferase activity (B).*** *Cells were incubated with increasing concentrations of glucagon in 96-well or in 384-well plates for 5 hours. ONE-Step™ Luciferase reagent was added for 15 minutes at room temperature (RT) and luminescence was measured in a luminometer. Results are expressed as fold induction, with luminescence of unstimulated cells set to 1.* ***Signal stability in luciferase reporter cell lines (C).*** *Cells were incubated with increasing concentrations of glucagon for the indicated times. ONE-Step™ Luciferase reagent was added for 15 minutes at RT and luminescence was measured in a luminometer. Results are expressed as percent of maximum activity, with maximum set to 100%.*

AGONIST STABILITY IN SERUM

Drug developers may need to examine the stability of candidate agonists in serum, since these compounds remain in the bloodstream upon administration to patients. Numerous factors contribute to the degradation of agonists within this environment. These experiments indicate that our reporter cell lines are suitable systems to perform such assays.

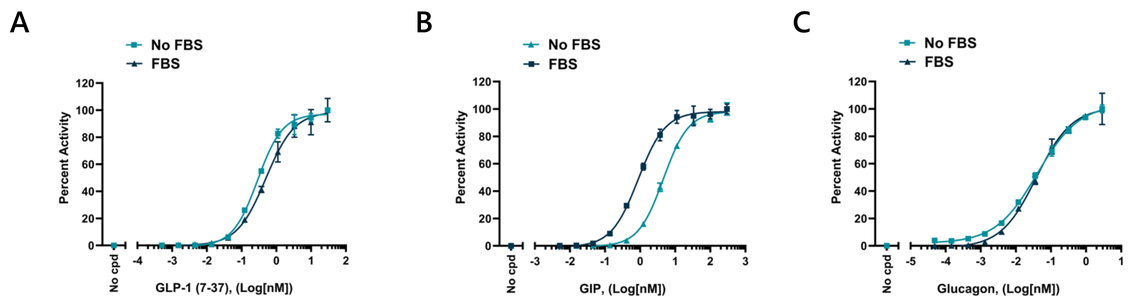

Figure 3. Agonist stability in serum. *Cells were incubated with increasing concentrations of GLP-1* ***(A),*** *GIP* ***(B),*** *or glucagon* ***(C)*** *for 5 hours. The agonist was diluted in medium containing 10% FBS (Fetal Bovine Serum) or in Opti-MEM™. ONE-Step™ Luciferase reagent was added for 15 minutes at room temperature and luminescence was measured in a luminometer. Results are expressed as percent of maximum activity, with maximum set to 100%.*

RESPONSES TO KNOWN AGONISTS

Reporter activation by incretin analogs was assessed by measuring luciferase activity. All analogs except for glucagon were assayed in GLP-1R/CRE cells. Tirzepatide, a dual-receptor agonist, and Retatrutide, a triple hormone receptor agonist, were assayed in GLP-1, GIPR, and Glucagon receptor cell lines. All cell lines display a strong increase in luciferase activity upon addition of the respective agonist. Sensitivity was in the range of 0.05 nM for GLP-1, 0.4 nM for GIP, and 0.004 nM for glucagon. Incubation times were optimized for each cell line to ensure signal stability when performing the bioassay.

Since the muscle loss experienced upon administration of GLP agonists may be reverted by Activin/Myostatin blockers, we also validated the inhibition of the Activin/Myostatin signaling pathway by inhibitors or receptor blockers.

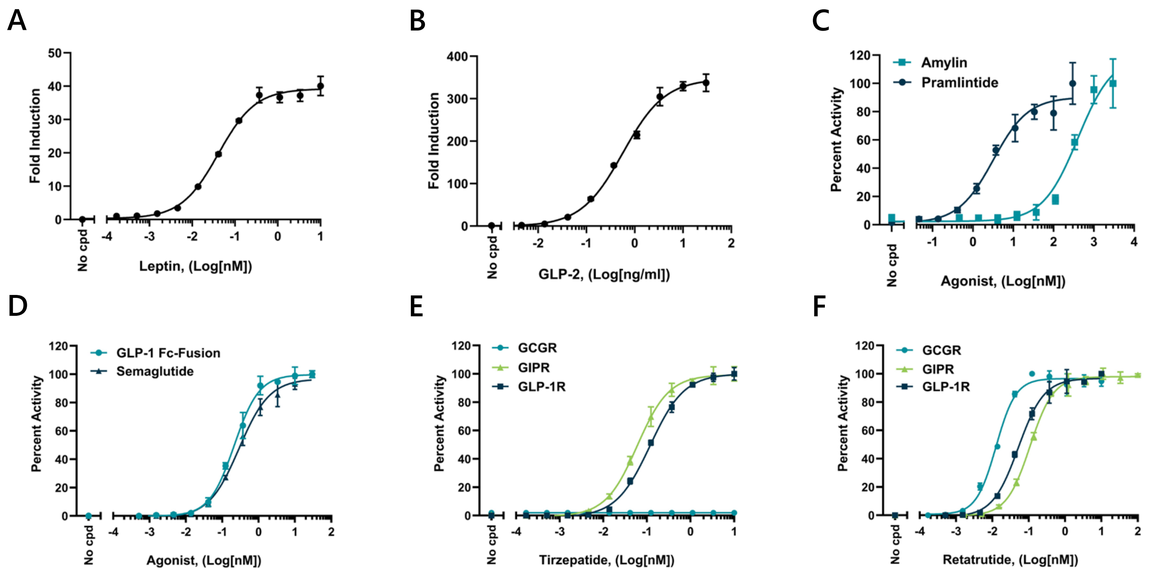

Figure 4. Comparison of analog potency in GLP-1R, GIPR, and GCGR/CRE Luciferase Reporter Cell Lines. *Cells expressing the corresponding receptor were incubated with increasing concentrations of agonist for 5 hours before addition of ONE-Step™ Luciferase reagent and luminescence measurement.* ***(A-C)*** *Results are shown for Leptin, GLP-2, and Amylin receptor-expressing cell lines;* ***(D)*** *GLP-1R agonists were assayed in the GLP-1R cell line;* ***(E)*** *Tirzepatide was assayed in all cell lines;* ***(F)*** *Retatrutide was assayed in all cell lines. Results are expressed as percent of maximum activity, with maximum set to 100%.*

Table 2: Analog EC_{50} in GLP-1R, GIPR, or GCGR/CRE Luciferase Reporter Cell Lines. *Luciferase activity resulting from incubation with known analogs was measured in GLP-1R, GIPR, and Glucagon receptor (GCGR) cells. Retatrutide and tirzepatide were assayed in the three cell lines. Not applicable (n/a) indicates that the analog is not known to activate this receptor and was not assayed. Where indicated, Standard Deviations (SD) of EC_{50} were calculated from 3 to 5 separate experiments, each run in triplicate (except GLP-1 Fc-fusion, for which n=2). For each condition, EC_{50} was determined by curve fitting using Prism software v 11.0.*

Analog	GLP-1R, EC_{50}	GIPR, EC_{50}	GCGR, EC_{50}
GLP-1 (7-37)	0.92 ± 0.86 nM	n/a	n/a
GIP	No stimulation	3.6 ±1.5 nM	n/a
Glucagon	n/a	n/a	0.03 ± 0.01 nM
Retatrutide	0.14 ± 0.14 nM	0.4 ± 0.5 nM	0.02 ± 0.02 nM
Tirzepatide	0.09 ± 0.02 nM	0.1 ± 0.1 nM	No stimulation
GLP-1 Fc-Fusion	0.25 ± 0.07 nM	n/a	n/a
Semaglutide	0.3 nM	n/a	n/a

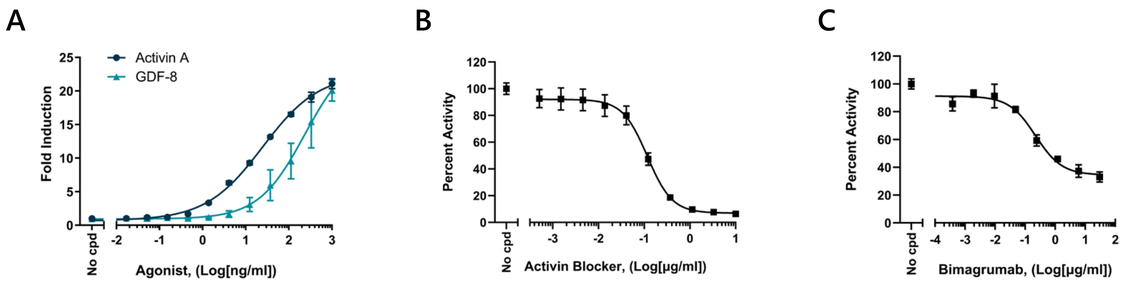

Figure 5. Validation of agonists and antagonists in the Activin/Myostatin Luciferase Reporter Cell Line. (A) *Activin/Myostatin Luciferase Reporter cells were stimulated with increasing concentrations of Activin A (PeproTech #120-14E) or GDF-8/myostatin (R&D Systems #788-G8) for 18 hours before addition of ONE-Step™ Luciferase reagent and luminescence measurement;* ***(B)*** *Cells were pre-incubated with increasing doses of Activin Blocker (#102121) prior to stimulation with 30 ng/ml of Activin A;* **(C)** *Cells were pre-incubated with increasing concentrations of bimagrumab (MedChemExpress #HY-P99355) prior to stimulation with 200 ng/ml GDF-8/Myostatin.*

SUMMARY & CONCLUSION

Cell-based assays are pivotal in drug development, enabling the assessment of potential drug candidates' biological activity on living cells and providing insight into their mechanisms. They offer a more precise depiction of compound interactions with living cells compared to biochemical assays. Inducible reporter assays, particularly luciferase reporters linked to pathway-specific promoters, offer a straightforward, robust, and quantitative way to measure signaling activity.

Single clone, stable HEK293 cell lines were generated to measure agonist activation of receptors for GLP-1, GLP-2, GIP, Glucagon, Activin/Myostatin, Leptin, and Amylin. Most of these cell lines overexpress the receptor of interest (Table 1) and a firefly luciferase reporter under the control of the appropriate response elements. Luciferase activity can be measured using a simple luminometer and is directly proportional to receptor activation.

All cell lines produced a robust luciferase readout upon stimulation of the cell surface receptor and are ideal for screening candidate molecules and determining their EC_{50}.

- Experimentally determined EC_{50} consistent with existing data
- **Strong induction signal**: up to 400-fold stimulation depending on the cell line
- **Stable signal**: luminescence maintained from 4 hours to 6 hours after analog addition
- **Amenable to high throughput**: similar results were obtained using 96-well and 384-well formats across all tested cell lines

	GLP-1	GIP	Glucagon	GLP-2	Leptin	Amylin	Activin A
EC_{50}	0.92 nM	3.6 nM	0.03 nM	0.574 ng/ml	0.041nM	402.6 nM	24.5 ng/ml

Table 3: Analog EC_{50} in all Luciferase Reporter cell lines. *Luciferase activity resulting from incubation with the indicated analogs was measured in the corresponding cell lines as shown in Figures 2-5.*

References

(1) Coskun T, *et al.* 2022, *Cell Metabolism* 34: 1234-1247.
(2) Yang B, *et al.* 2022, *Molecular Metabolism* 66: 101638.
(3) Zhao X, *et al.* 2021, *Front. Endocrinol.* 12: 721135.

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