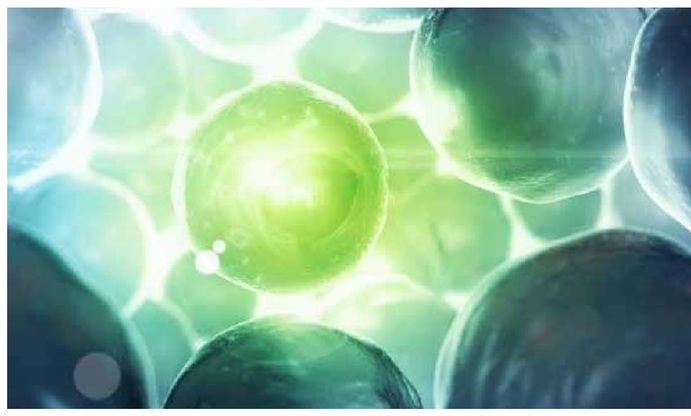
FGF21 Responsive Reporter Gene Cell Line With Improved Sensitivity



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Abstract

A FGF21 responsive reporter cell line has been established that exhibits enhanced sensitivity to treatment with FGF21 by replacing the FGFR1c co-receptor β-Klotho with a codon optimized synthetic β-Klotho gene. This cell line allows the precise and rapid quantification of FGF21 activity even in the presence of human serum.

Introduction

Human fibroblast growth factor-21 (FGF21) has the potential to improve glycemic control in patients with type 2 diabetes, as well as the control of weight gain. Analogues of FGF21 are currently being tested in clinical trials in patients with type 2 diabetes and there is a need for a specific assay with enhanced sensitivity both for the quantification of the potency and neutralizing antibody response to FGF21 and related analogues in human serum. A reporter gene cell line has been established that exhibits a high degree of sensitivity to treatment with human FGF21 even in the presence of human serum. These cells can be used in a frozen assay-ready format that confers ease of use for both potency assays and neutralizing antibody assays for monitoring patients treated with FGF21 related products.

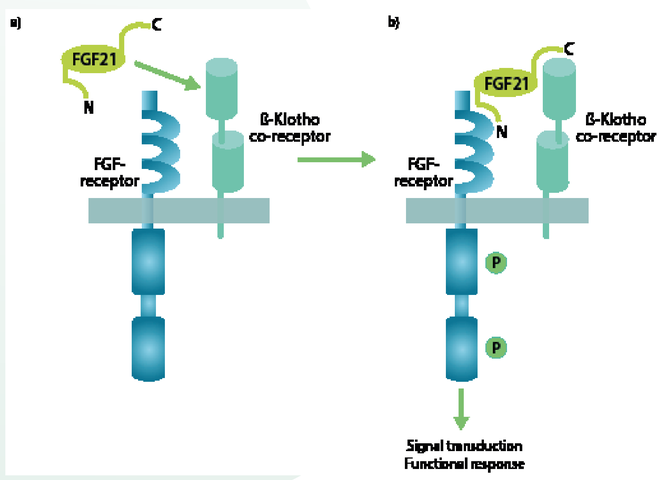

Illustration: *Schematic illustration of FGF21 binding to FGF receptor 1c (FGFR1c) in the presence of the codon optimized co-receptor β-Klotho.*

Methods

Human HEK293 or Jurkat cells were co-transfected sequentially, with a *Firefly* luciferase (FL) reporter-gene construct regulated by a chimeric transcription factor consisting of the trans-activation domain of Elk-1 fused to the GAL4 DNA binding domain (**Figure 1A**) together with an expression vector for the chimeric transcription factor. Since the GAL4 DNA binding domain does not exist in mammalian cells, only the chimeric transcription factor will bind to the up-stream activation sequence (UAS) of GAL4 regulating transcription of the FL reporter gene. The cells were also co-transfected with the gene encoding *Renilla* luciferase under the control of a constitutive promoter, used to normalize FGF21 induced FL activity, and the FGFR1c receptor chain together with a codon optimized synthetic co-receptor β-Klotho gene with 79% homology to the native gene (**Figure 1B**).

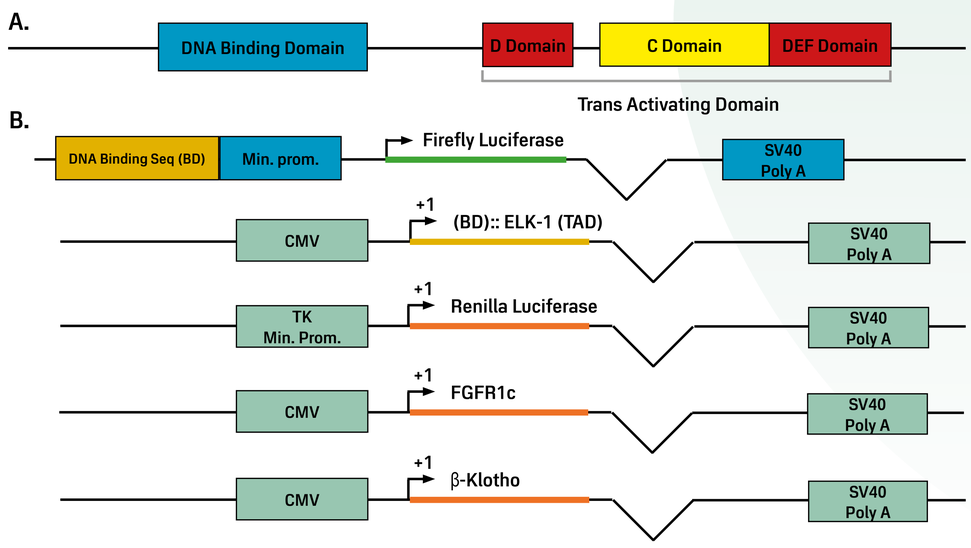

Figure 1.
A: *Structure of the chimeric transcription factor (BD)::Elk-1 (TAD)*
B: *Expression vectors introduced into HEK293 cells*

Results

I. Assay-Ready HEK293 cells with improved sensitivity to FGF21

Three different FGF21 responsive reporter cell lines (Jurkat, HEK293 native β-Klotho, and HEK293 optimized β-Klotho) produced in a frozen assay-ready format were thawed and incubated with increasing concentrations of FGF21 at 37°C, for 6 hrs prior to quantification of firefly luciferase activity. HEK293 cells transfected with the optimized β-Klotho gene exhibited enhanced sensitivity relatively to either HEK293 cells or Jurkat cells transfected with the native β-Klotho gene as can be seen in **Table 1**.

	HEK293 Optimized β-Klotho	HEK293 Native β-Klotho	Jurkat Native β-Klotho
Top (RLU)	**142702**	130408	40903
Bottom (RLU)	**13685**	1737	1530
Hill Slope	**1.216**	1.142	1.002
EC50 (ng/mL)	**7.055**	23.97	171.6
Span (RLU)	**129017**	128670	39373

Table 1. *Key characterizations of three FGF21 responsive cell lines produced in an frozen assay-ready format. Sigmoidal, 4PL, Best-fit values.*

II. The response of individual vials of frozen HEK293 cells (optimized β-Klotho) to treatment with FGF21

Vials of frozen assay-ready HEK293 cells (optimized β-Klotho) were thawed and treated with increasing doses of FGF21 for 6 hours at 37°C, prior to the quantification of luciferase activity using Dual Glo (Promega, Catalogue N° 2920) as shown in **Figure 2**. The assay-ready format confers ease of use while maintaining a high degree of sensitivity and specificity comparable to that of cells in culture.

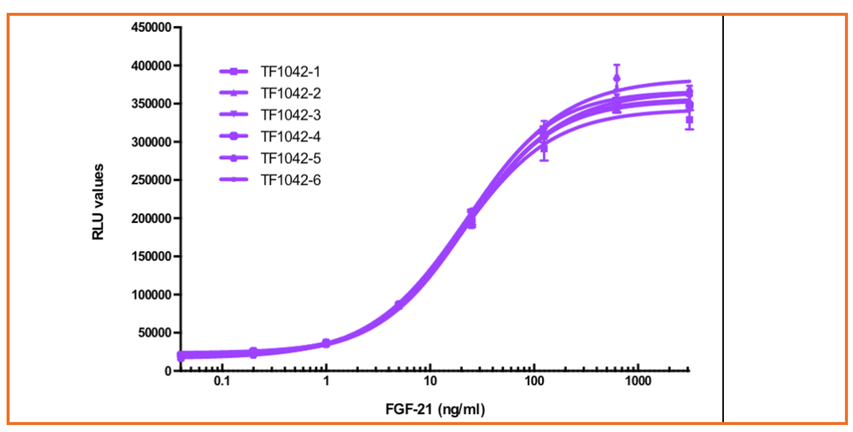

Figure 2. *Response of individual vials of assay-ready HEK293 (optimized β-Klotho) cells to treatment with FGF21*

III. Normal human sera have a minimum effect on the response of Assay-Ready Frozen HEK293 cells (optimized β-Klotho) to treatment with FGF21

Frozen assay-ready HEK293 cells (optimized β-Klotho) were thawed and treated with increasing concentrations of FGF21 in the presence of a 1/20 final dilution of human sera from normal doners at 37°C, for 6 hours prior to quantification of firefly luciferase activity. (**Figure 3**). The response of FGF21 is only minimally affected by the presence of normal human sera, suggesting that the use of a chimeric transcription factor to regulate reporter gene expression markedly limits interference from other growth factors and cytokines present in normal human serum.

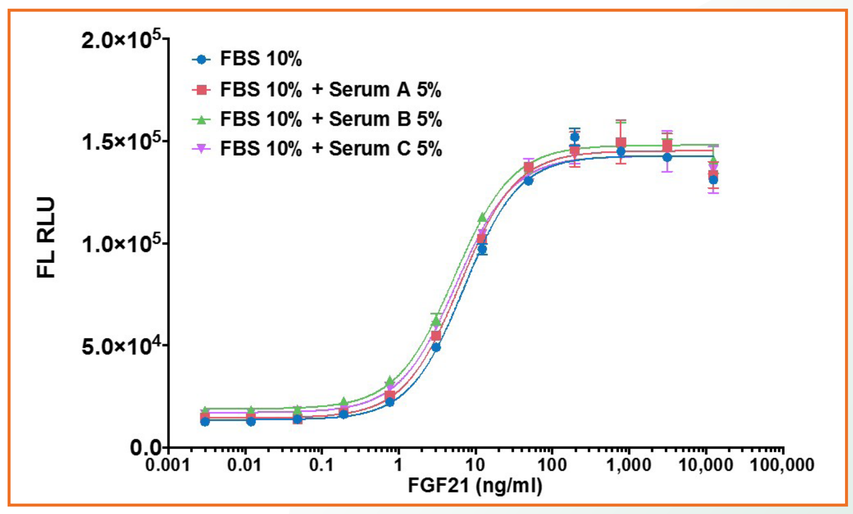

Figure 3. *Effect of normal human sera on the response of Assay-Ready HEK293 cells (optimized β-Klotho) to treatment with FGF21*

Conclusions

A Human β-Klotho gene was designed to use the codons most frequently employed statistically in order to optimize translation and hence increase the efficiency with which the β-Klotho co-receptor facilitates binding of FGF21 to its receptor and activation of signal transduction.

Synthesis of the corresponding gene and its use to transfect human HEK293 cells led to the establishment of a cell line that is markedly more sensitive than FGF21-responsive Jurkat or HEK293 cell lines previously established using the same cloning strategy but employing the native β-Klotho gene.