FGF21 Responsive Reporter Gene Cell Line With Improved Sensitivity

Lue Huang, Christophe Lallemand, Feifei Liang, Flore Staub, and Michael G. Tovey
Biomonitor SAS, Villejuif Bio Park, 1 Mail du Professeur Georges Mathé 94800 Villejuif France

Abstract
A FGF21 responsive reporter cell line has been established that exhibits enhanced sensitivity to treatment with FGF21 by replacing the FGF1c 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.

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 upstream 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 FGR1c receptor chain together with a codon optimized synthetic co-receptor β-Klotho gene with 79% homology to the native gene (Figure 1B).

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 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.

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° 2100) 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.

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 serum from normal donors at 37°C, for 6 hrs 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.

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.