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A Screening Method of a Material Inducing Klotho Transcripitional Activation

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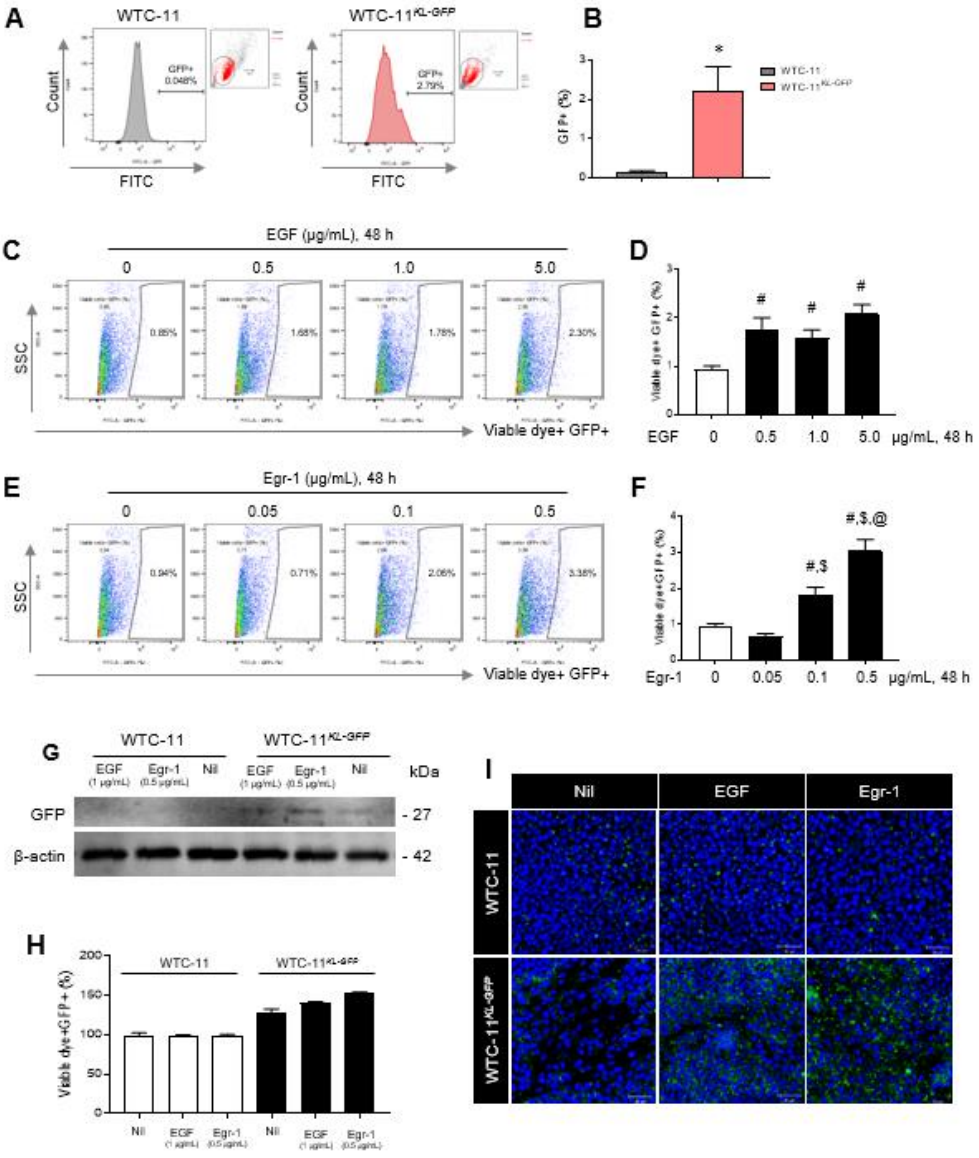
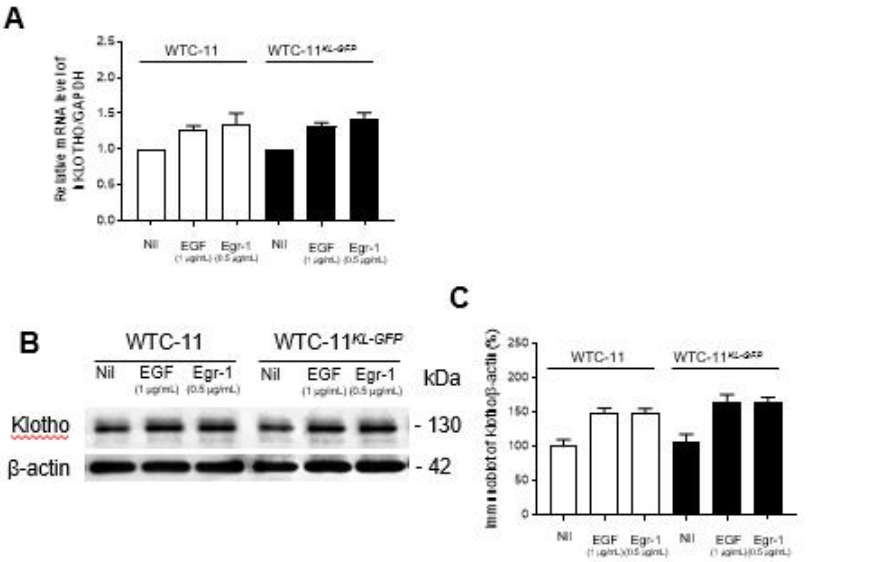
Objectives : Klotho is a novel protein encoded by the KL gene, located on human gene 13. Klotho is highly expressed in the kidneys and brain, and less so in other organs, and is also detected in cerebrospinal fluid and urine. In addition, Klotho is associated with several cellular processes related to aging. The aim of this study relates to a method of screening for substances that activate transcription of the Klotho gene by knock-in fluorescent proteins into the Klotho gene of a cell using CRISPR/Cas9 technology.
Methods : To insert the green fluorescent protein (GFP) DNA sequence into the 3'-UTR position of the human Klotho gene, a donor construct knocked in by adding a homology arm to the 3'-UTR position of the human KLOTHO gene, which flacks the GFP gene and hygromycin to IRES. Next, the above construct was inserted into the target position by CRISPR/Cas9 technology. Flow cytometry, fluorescent staining and protein immunoblotting are used to determine whether the target gene is functioning properly.
Results : Sanger sequencing confirmed that the GFP gene was accurately inserted into the target site. The hiPSCs of WTC-11KL-GFP showed typical pluripotency markers and normal karyotype. Flow cytometry showed a nearly twofold increase in GFP-positive cells in the edited hiPSC line compared to controls. Immunoblotting results showed that knock-in of the GFP gene had no effect on the Klotho inducible substance itself, Comparison of the fluorescent staining of WTC-11$^{KL\text{-}GFP}$ and WTC-11 shows that the fluorescence of the former under confocal observation reflects a well-functioning GFP gene knocked into the Klotho gene.
Conclusions : Knock-in fluorescent proteins into the Klotho gene in cells can be used to screen for substances that activate the transcription of the Klotho gene.

1.png


A
WTC-11
WTC-11KL-GFP
Count
FITC
B
GFP+ (%)
WTC-11
WTC-11KL-GFP
C
EGF (μg/mL), 48 h
0
0.5
1.0
5.0
SSC
Viable dye+ GFP+
D
Viable dye+ GFP+ (%)
EGF 0 0.5 1.0 5.0 μg/mL, 48 h
E
Egr-1 (μg/mL), 48 h
0
0.05
0.1
0.5
SSC
Viable dye+ GFP+
F
Viable dye+GFP+ (%)
Egr-1 0 0.05 0.1 0.5 μg/mL, 48 h
G
WTC-11
WTC-11KL-GFP
EGF
Egr-1
Nil
kDa
GFP
β-actin
- 27
- 42
H
WTC-11
WTC-11KL-GFP
Viable dye+GFP+ (%)
Nil
EGF
Egr-1
I
Nil
EGF
Egr-1
WTC-11
WTC-11KL-GFP

1.png


A
WTC-11
WTC-11KL-GFP
Nil
EGF
Egr-1
B
kDa
Klotho
β-actin
- 130
- 42
C
D
DAPI
KLOTHO
Merge