Poster P1 - 593

William Harvey Research Institute

The mechanistic role of Fibroblast growth factor 21 (FGF21) in Growth Hormone resistance secondary to chronic childhood conditions

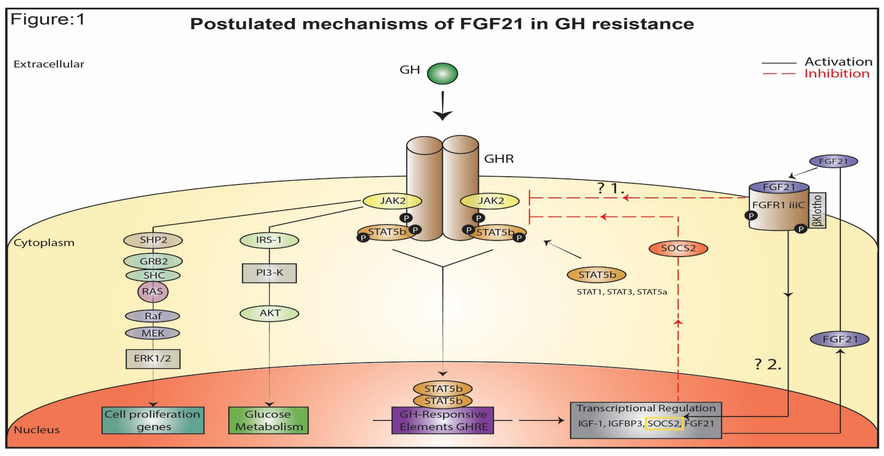
Jayna Narendra Mistry[1], Gerard Ruiz-Babot[1], Farasat Zaman[2] Lars Sävendahl[2], Leonardo Guasti[1] & Leo Dunkel[1]

[1]Centre for Endocrinology, William Harvey Research Institute, Queen Mary University of London (QMUL), London (UK)
[2]Department of Women's and Children's Health, Karolinska Institutet and University Hospital, Stockholm (Sweden)

Introduction

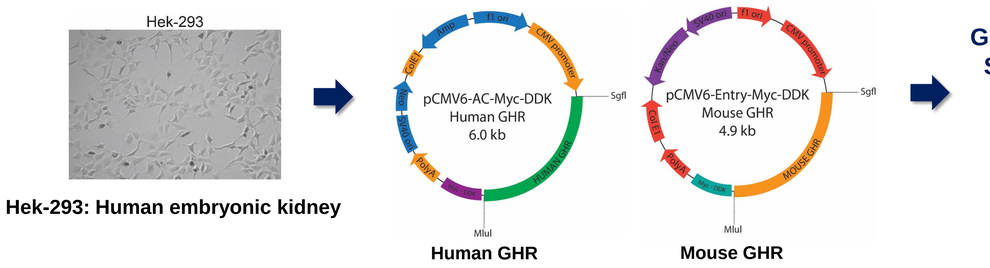
Undernutrition and chronic inflammation is known to impair linear growth through resistance to GH [1]. Fibroblast growth factor 21 (FGF21); a member of a subfamily of FGFs (including FGF15/19 and FGF23) is considered an important regulator of the metabolic adaptation to fasting, inducing gluconeogenesis, fatty acid oxidation and ketogenesis. The activation FGF21 is highly dependent on the interaction of specific receptors (β-Klotho/ FGFR1 iiiC), forming a complex with FGF21 on the cell surface [2]. Recent studies have shown that elevated expression of FGF21, secondary to prolonged undernutrition develops GH resistance and subsequent attenuation of skeletal growth and growth plate chondrogenesis in both mice and human (Fig.1) [1]. Molecular understanding of this process may open avenues for novel therapeutic intervention to enhance linear growth of children with secondary GH resistance.

Figure:1



Objective: To unravel the mechanistic interplay of FGF21 in GHR signaling.

Method

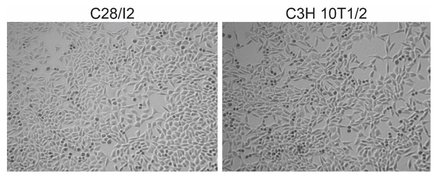


GENERATION OF STABLE LINES

Hek-293 hGHR: Human GHR

Hek-293 mGHR: Mouse GHR

CHONDROCYTE CELL LINES



C28/I2: Human costal chondrocytes

C3H 10T1/2: Mouse embryonic mesenchymal

EXPERIMENTAL DESIGN & SPECIFIC AIMS

Stable Lines
Hek-293 hGHR
Hek-293 mGHR

Chondrocyte cell lines
C28/I2
C3H 10T1/2

Validation of the GHR model
- Assessment of GHR signaling.
- Expression of FGF21 receptor complex *in vitro* and *in vivo*.

The role of FGF21 in GH resistance
- Determine the role of FGF21 on GHR half-life.
- Examine the affect of FGF21 on JAK/STAT signaling and negative feedback regulation SOCS2.

Results

Expression of GHR in stable line model

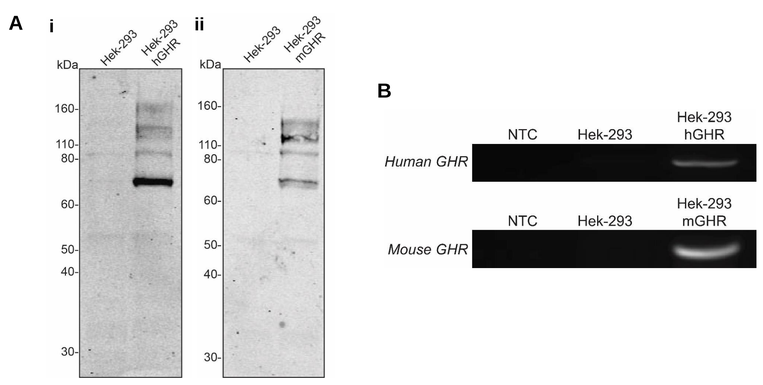


Figure 2: Establishment of HEK-293 GHR expressing stable lines and confirmation of pattern levels. Hek-293 cells were non-transfected (control) or transfected with plasmids pCMV6-Entry-Myc-DDK (mouse GHR) or pCMV6-AC-Myc-DDK (Human GHR) with PEI as a transfection reagent to generate stable lines. **(A)** Western blot analysis of GHR (precursor GHR 110kDa, glycosylated mature GHR 140kDa) in stable lines **(i)** Hek-293 (control), Hek-293 human GHR (Hek-293 hGHR), **(ii)** (Hek-293 (control), Hek-293 mouse GHR (Hek-293 mGHR). **(B)** RT-PCR analysis of Human GHR and Mouse GHR expression in stable lines.

Growth hormone activates phosphorylation of STAT5

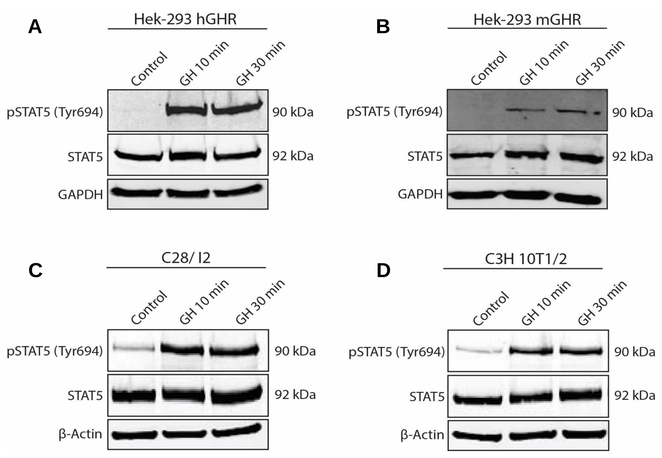


Figure 3: Functional analysis of GH activation on JAK/STAT signaling events. Hek-293 hGHR **(A)**, Hek-293 mGHR **(B)**, C28/I2 **(C)** and C3H 10T1/2 **(D)** cells were incubated in the absence or presence of GH (500ng/ml) for 10 or 30 minutes before analysis of STAT5 and phosphorylated STAT5 by western blot.

Expression of the FGF21 receptor complex repertoire in stable lines

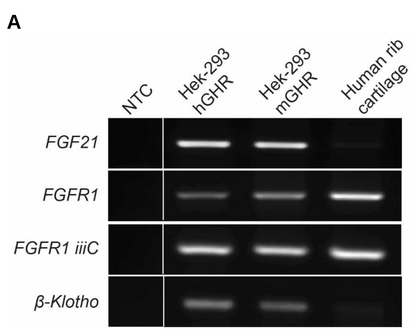
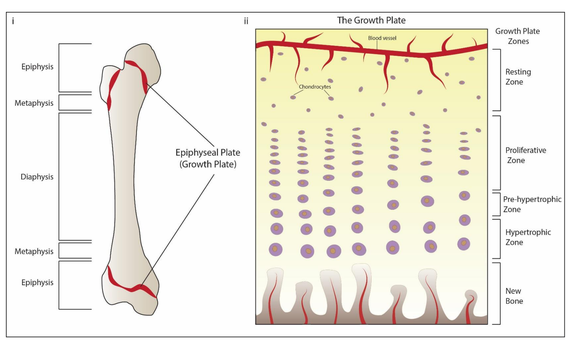


Figure 4: Human and Mouse GHR stable lines express the FGF21 receptor complex. **(A)** Assessment of the FGF21 receptor complex (FGF21, FGFR1, FGFR1 iiiC and β-Klotho) in Hek-293 hGHR, Hek-293 mGHR and human rib cartilage (positive control) using RT-PCR.

FGF21 receptors are predominantly expressed within the proliferative and pre-hypertrophic zones



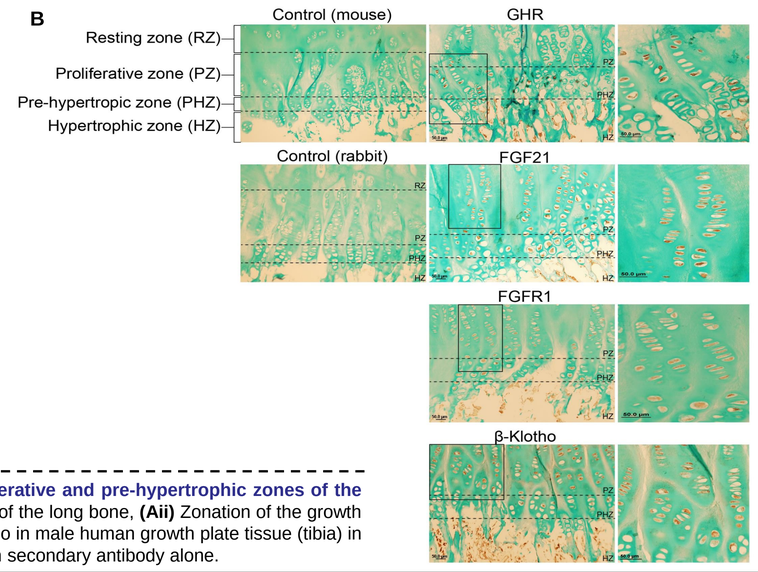


Figure 5: FGF21 receptors; FGFR1 and β-Klotho are localised in the proliferative and pre-hypertrophic zones of the human growth plate. **(A)** Illustration of growth plate development, **(Ai)** Regions of the long bone, **(Aii)** Zonation of the growth plate. **(B)** Immunohistochemical localisation of GHR, FGF21, FGFR1 and β-Klotho in male human growth plate tissue (tibia) in late puberty. Negative controls for human growth plate tissue were incubated with secondary antibody alone.

Chronic exposure to FGF21 reduces GHR half-life

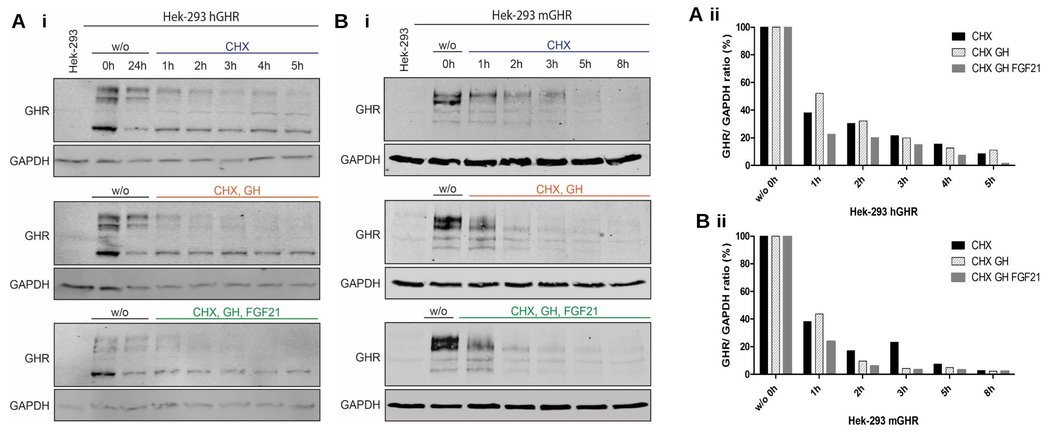


Figure 6: The effect of GH and FGF21 on GHR turnover. Hek-293 hGHR **(Ai, Aii)** and Hek-293 mGHR **(Bi, Bii)** were treated in the absence or presence of Cycloheximide (CHX), GH (500ng/ml) or recombinant human/ mouse FGF21 (5µg/ml) for 1 – 8h before analysis of GHR by western blot.

Chronic exposure to FGF21 reduces phosphorylation of STAT5

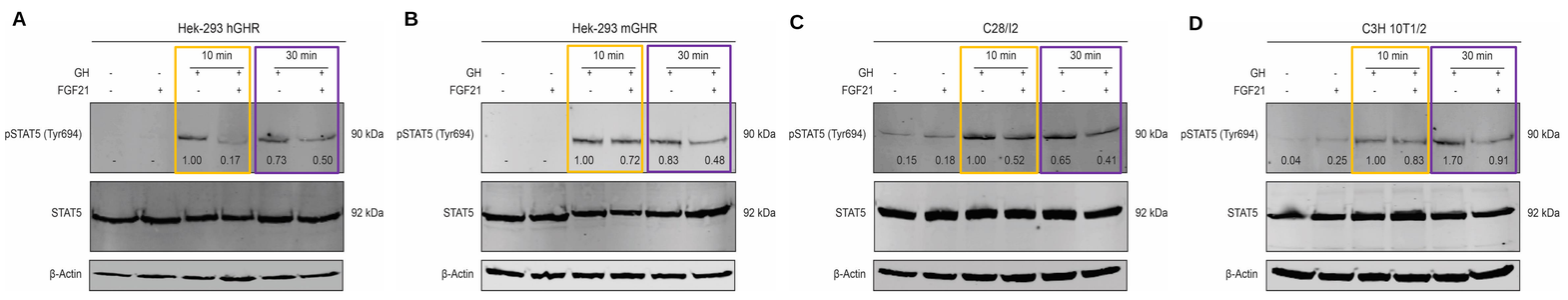


Figure 7: The effect of GH and FGF21 on JAK/STAT signaling. Hek-293 hGHR **(A)**, Hek-293 mGHR **(B)**, C28/I2 **(C)** and C3H 10T1/2 **(D)** cells were untreated or incubated overnight with recombinant human/ mouse FGF21 (5µg/ml). 24h later cells were challenged in the absence or presence of GH (500ng/ml) for 10 or 30 minutes before analysis of STAT5 and phosphorylated STAT5 by western blot.

Chronic exposure to FGF21 increases SOCS2 expression

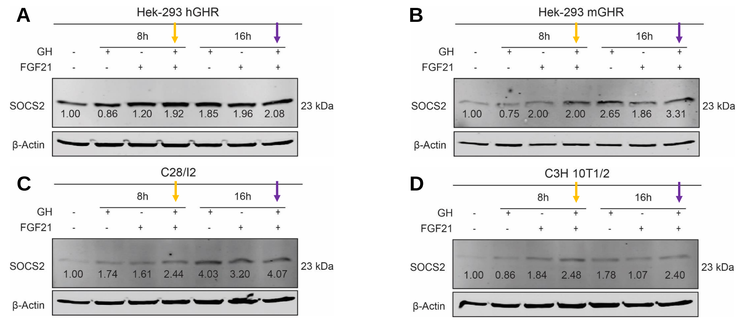


Figure 8: The effect of GH and FGF21 on SOCS2 negative feedback regulation. Hek-293 hGHR **(A)**, Hek-293 mGHR **(B)**, C28/I2 **(C)** and C3H 10T1/2 **(D)** were treated in the absence or presence of GH (500ng/ml) and /or recombinant human/ mouse FGF21 (5µg/ml) for 8 or 16 hours before analysis of SOCS2 expression by western blot.

Conclusion

Validation of the GHR model

- **Generated the tools to study GH/GHR signaling in stable cell lines and chondrocyte cell lines.**
- **Growth hormone potentiates the activation of down-stream signaling in the JAK/STAT5 pathway.**

The proposed mechanism of FGF21 in GH resistance

- **Chronic exposure to FGF21 reduces GHR half-life and inhibits early upstream mediators (pSTAT5) in GHR signaling.**
- **Chronic exposure to FGF21 increases SOCS2 expression.**

References

[1] Guasti, L., Silvennoinen, S., Bulstrode, N.W., Ferretti, P., Sankilampi, U. and Dunkel, L. Elevated FGF21 leads to attenuated postnatal linear growth in preterm infants through GH resistance in chondrocytes. *J Clin Endocrinol Metab*. 2014. 99(11), E2198-206.
[2] Angelin, B., Larsson, T.E. and Rudling, M. Circulating fibroblast growth factors as metabolic regulators – a critical appraisal. *Cell Met.* 2012. 16(6): 693-705.

Disclosure Statement

I confirm that I do not have any conflict of interest in this study.

Jayna Narendra Mistry
j.n.mistry@qmul.ac.uk
+44 (0)207 882 6241

55ESPE 593--P1
Growth
Jayna Mistry
DOI: 10.3252/pso.eu.55ESPE.2016
Poster presented at: ESPE 2016 PARIS