The role of β-TrCP as a compensatory negative regulator in the successful GH/GHR and EGF/EGFR signalling in GHTD.

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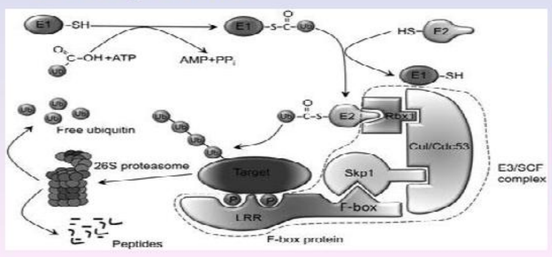
INTRODUCTION

The authors have nothing to disclose

- **GHTD is characterized by:**
  - over-expression of the E3 ubiquitin ligase, CIS.
  - excessive GHR endocytosis and degradation
  - impaired STAT3 phosphorylation[1]
- Induction of GHTD fibroblasts with 200μg/L hGH (GH200) and silencing mRNA CIS (siCIS) or with high dose of hGH, 1000 μg/L (GH1000), suppresses excessive CIS and restores normal GH signalling.
- Crosstalk between the GH and EGF signalling pathways is important for normal cellular development. [2]
- The ubiquitin ligase SCF^{TrCP} is required for internalisation of the growth hormone receptor (GHR).[3]
- β-transducin repeat-containing protein (β-TrCP), the F-box protein of the E3 ubiquitin ligase SCF, also plays a role in GHR endocytosis.[3]



The ubiquitin ligase system : β-TrCP is the substrate recognition subunit of the E3 ligase $SCF^{β\text{-}TrC}$

OBJECTIVE

To study the role of β-TrCP in the negative regulation of the GH/GHR and EGF/EGFR pathways in normal and GHTD cells.

METHODS

- Fibroblast cultures were developed from gingival biopsies of 1 GHTD patient and 1 control child.
- The protein expression and the cellular localization of β-TrCP were studied by Western Immunoblotting and Immunofluorescence, respectively:

a) At the basal state and after induction with 200 μg/L hGH (GH200), either with or without siRNA CIS.

b) At the basal state and after inductions with 200 μg/L hGH (GH200), 1000 μg/L hGH (GH1000) or 50 ng/ml EGF.

RESULTS

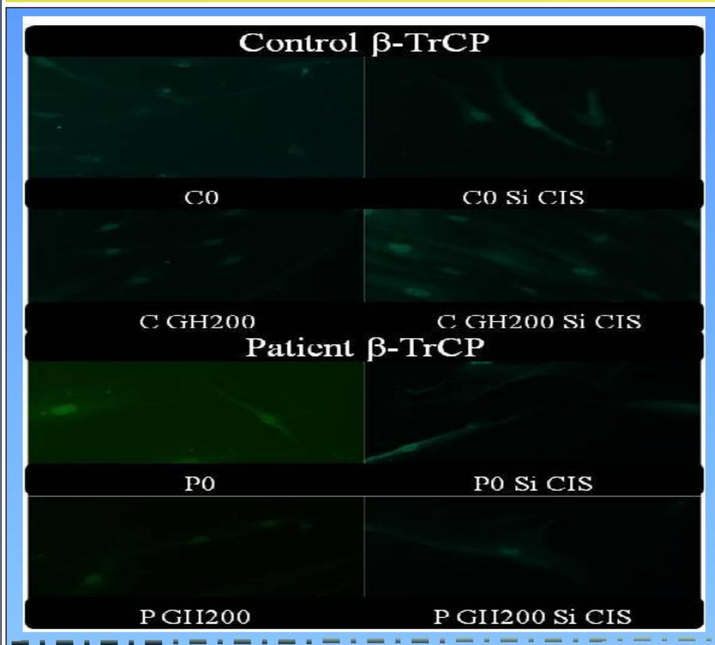


Figure 1.

After GH200/siCIS, the protein expression and cytoplasmic-membrane localization of β-TrCP were increased in the control and in the patient.

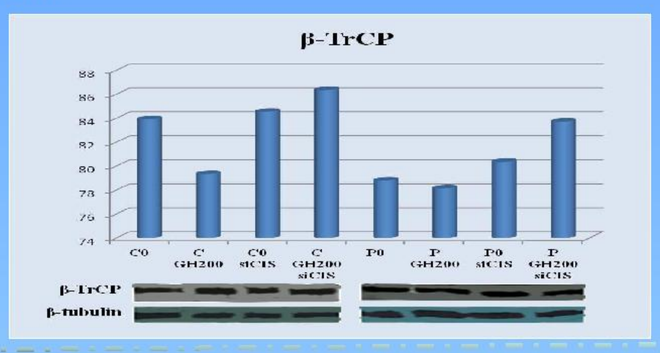
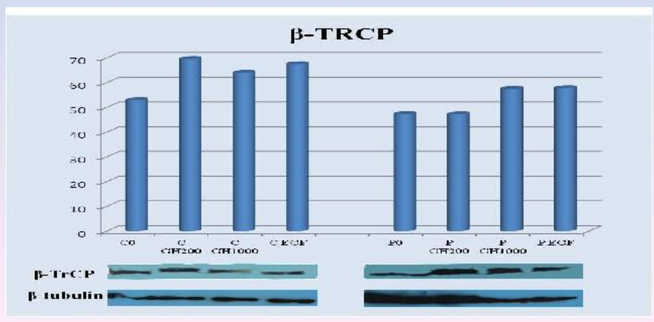
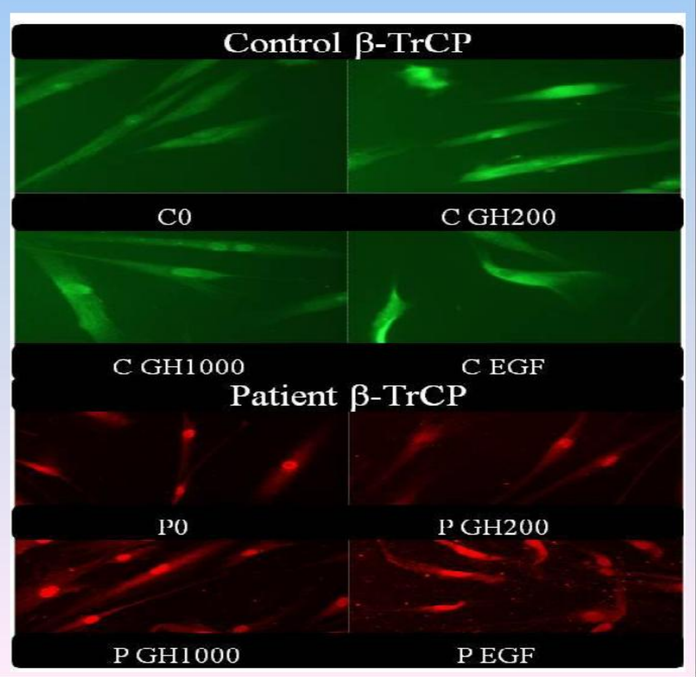


Figure 2.

- After induction with GH200 in the control and GH1000 in the patient (***inductions of successful GH signalling***), the protein expression and cytoplasmic-membrane localization of β-TrCP were increased.
- After induction with EGF, the protein expression and cytoplasmic-membrane localization of β-TrCP were also increased in both the control and the patient.





CONCLUSIONS

- When CIS is reduced, either after silencing of the CIS gene or after inductions of successful GH signalling, β-TrCP is increased and this may reflect a compensatory mechanism of negative regulation of the GH/GHR pathway in the control's and the patient's fibroblasts.
- β-TrCP also seems to participate in the negative regulation of the EGF/EGFR pathway in the control's and the patient's fibroblasts.
- β-TrCP seems to be activated more readily in the control's fibroblasts than in the GHTD's fibroblasts after inductions of successful GH and EGF signalling.

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