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

Eirini Kostopoulou¹, Andrea Paola Rojas-Gil², Alexia Karvela¹, Bessie E. Spiliotis¹

1. Pediatric Endocrine Research Laboratory, Division of Pediatric Endocrinology and Diabetes, Department of Pediatrics, University of Patras School of Medicine, Patras, Greece.
2. Faculty of Human Movement and Quality of Life Sciences, Department of Nursing, University of Peloponnese.

INTRODUCTION

The authors have nothing to disclose

β-TrCP is characterized by:
- over-expression of the E3 ubiquitin ligase, CIS.
- excessive GHR endocytosis and degradation
- impaired STAT3 phosphorylation
- 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.
- The ubiquitin ligase SCFβ-TrCP is required for internalisation of the growth hormone receptor (GHR).
- β-transducin repeat-containing protein (β-TrCP), the F-box protein of the E3 ubiquitin ligase SCF, also plays a role in GHR endocytosis.

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 Immuno blotting 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.

REFERENCES