The involvement of the Epidermal Growth Factor Receptor (EGFR) in the successful Growth Hormone (GH) signalling and the role of p21 in the negative regulation of the GH/GHR and EGF/EGFR pathways, in Growth Hormone Transduction Defect (GHTD).

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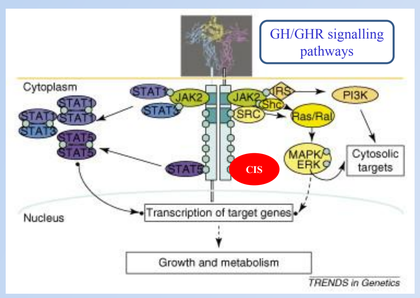
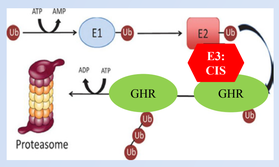
Introduction

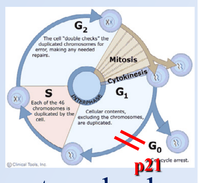
The authors have nothing to disclose

GHTD is characterized by:

- ✓ severe short stature and "catch-up" growth after hGH treatment.
- ✓ impaired STAT3 phosphorylation
- ✓ overexpression of the E3 ubiquitin ligase, CIS



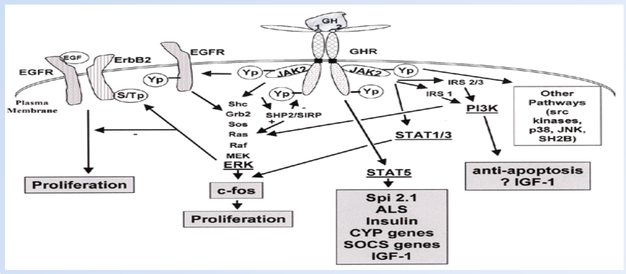




- ✓ overexpression of the negative regulator of cell cycle, p21.

STAT3 phosphorylation is restored by simultaneous induction of GHTD fibroblasts with 200μg/L GH and gene silencing of CIS (GH200/siRNA) or with 1000μg/L GH (GH1000) alone.[1]

Crosstalk between the GH and EGF pathways has been described in literature. [2,3,4]



Objective

The involvement of the EGFR in successful GH signalling and the role of p21 in the negative regulation of the GH/GHR and EGF/EGFR pathways in GHTD.

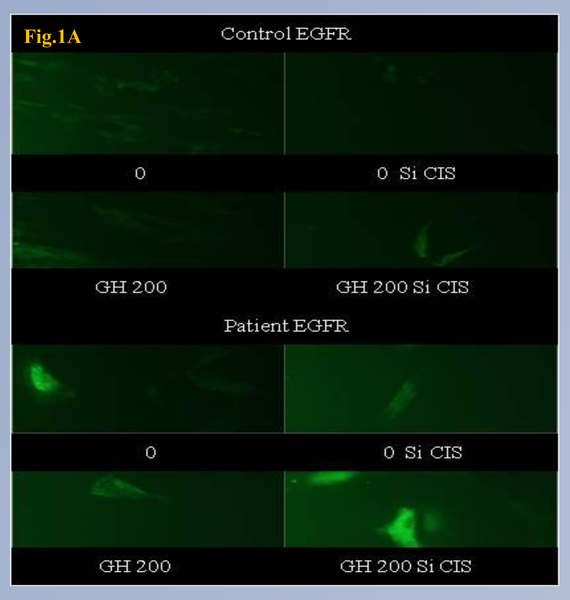
Methods

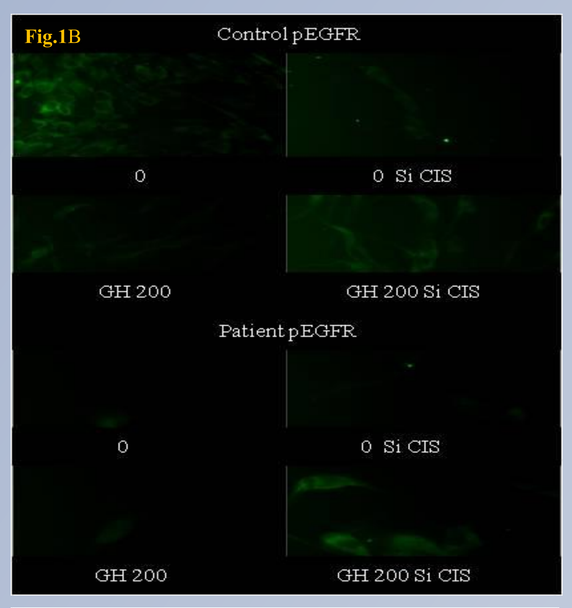
- Fibroblast cultures were developed from gingival biopsies of 1 GHTD patient and 1 control.
- The protein expression and cellular localization of EGFR, pEGFR and p21 were studied by Western Immunoblotting and Immunofluorescence, respectively:

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

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

Results





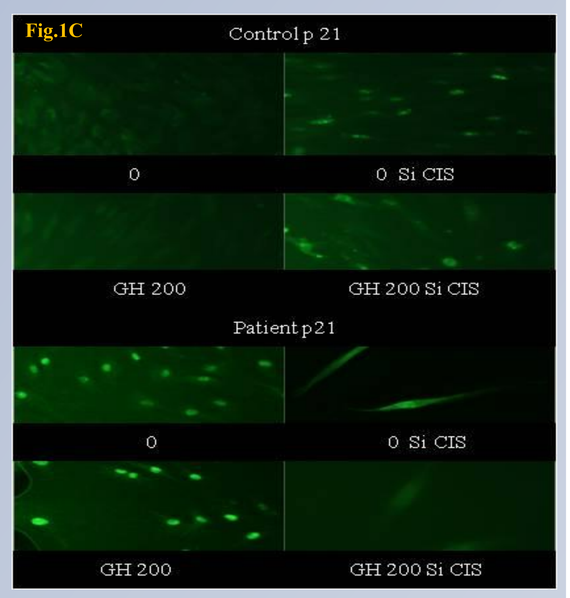
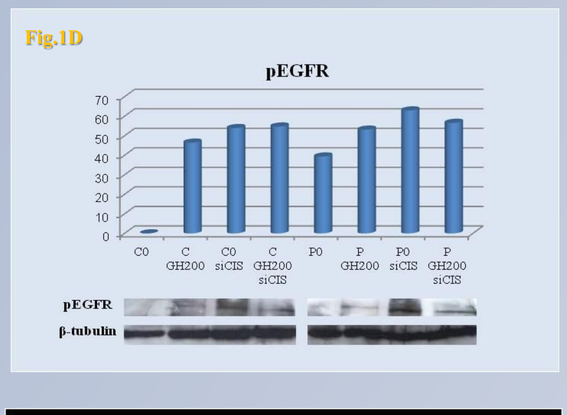
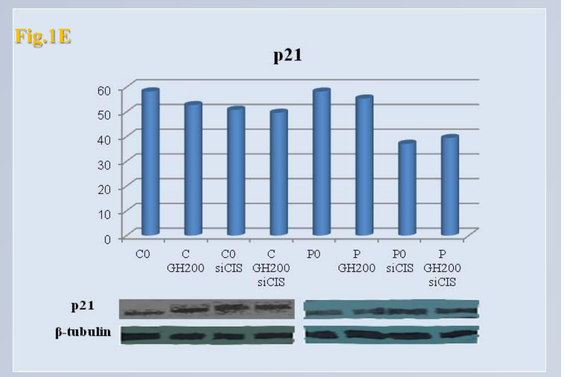
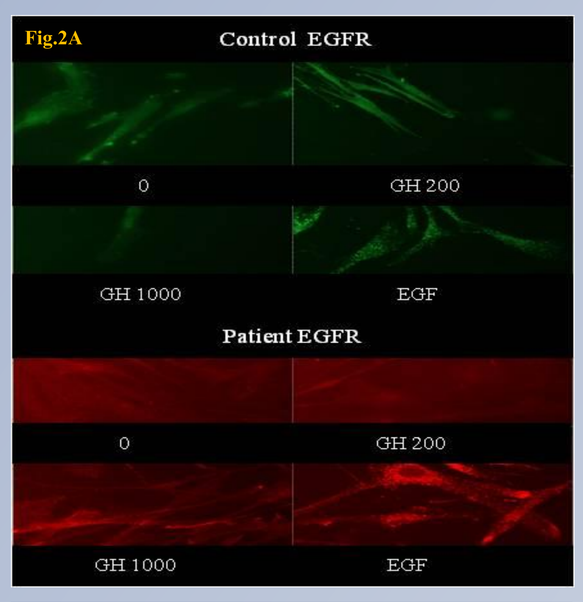
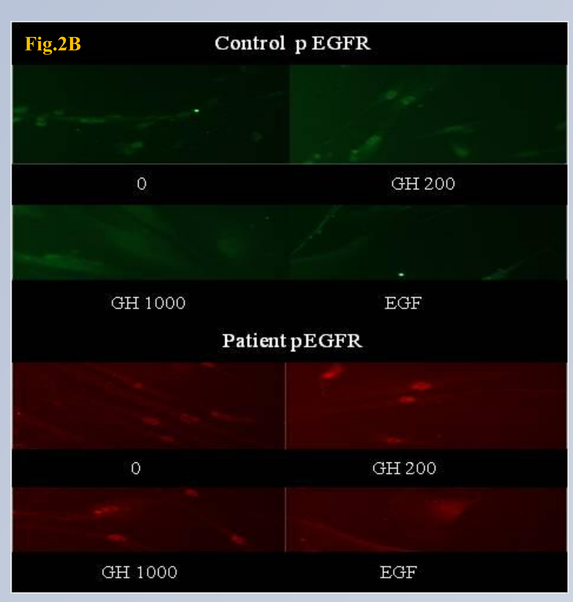


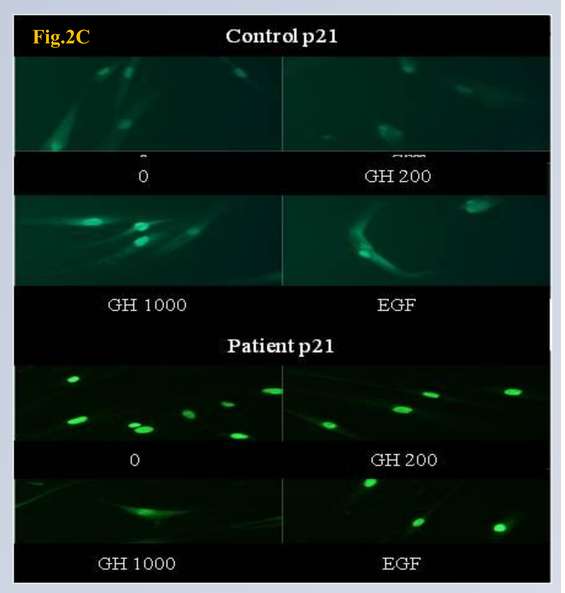
Figure 1. After GH200/siCIS: **i)** the protein expression and the membrane localization of EGFR (1A) and pEGFR (1B & 1D) were increased, especially in the patient, **ii)** the protein expression and the nuclear (anti-proliferative) localization of p21 were reduced in the patient (1C & 1E) .

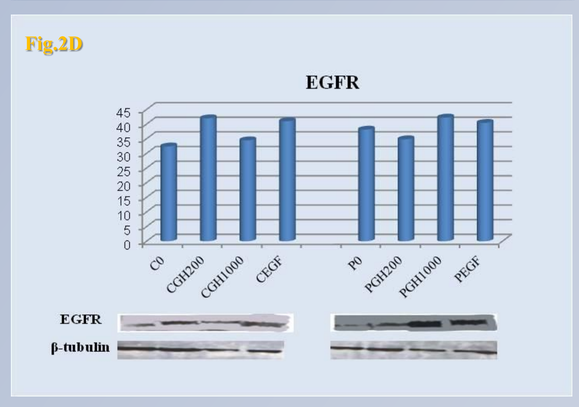


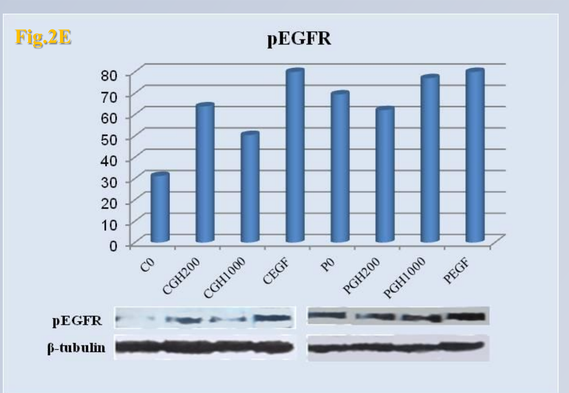












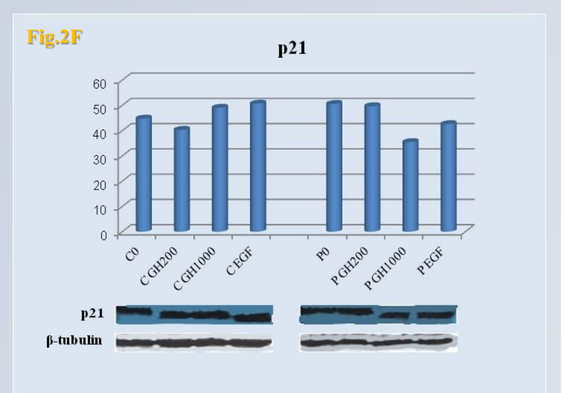


Figure 2.

- After the inductions where successful GH signalling was achieved (GH200 for the control and GH1000 for the patient):
  - i. the protein expression and the membrane localization of EGFR (2A & 2D) and pEGFR (2B & 2E) were increased and
  - ii. the protein expression and nuclear localization of p21 (2C & 2F) were reduced,
- After induction with EGF:
  - i. the protein and membrane expression of EGFR and pEGFR (2A, 2B, 2D & 2E) were increased similarly in the control and in the patient and
  - ii. The protein expression of p21 (2F)was increased in the control and reduced in the patient compared to the basal state.

Conclusions

In the fibroblasts of the GHTD patients:

1) When CIS is reduced, either after silencing of the CIS gene or after a higher dose of hGH (GH1000), the EGFR is activated, nuclear p21 (anti-proliferative effect) is reduced and GH signalling is successful.

2) The EGFR is involved in successful GH signalling, more intensely than in the fibroblasts of the control child. The EGFR may be involved in the mechanism of "catch-up" growth seen in the GHTD patients when exogenous hGH is administered.

3) p21 seems to participate also in negative regulation of the EGF/EGFR pathway in the control.

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