Supplemental Materials and Methods

In Situ Detection of DNA Fragmentation

Nick-end labeling was detected using the TUNEL technique by applying an in situ cell death detection kit following the manufacturer’s instructions (POD; Roche Molecular Biochemicals, Mannheim, Germany). Briefly, GH3B6 cells fixed in 4% formaldehyde were incubated with blocking solution for 10 min at RT, permeabilized with 0.1% Triton X-100 on ice and then incubated with TUNEL reaction mixture, containing enzyme (TdT) and nucleotides, in a humidified chamber at 37°C for 1 h. Finally, the convertor POD (secondary antibody conjugated with horseradish peroxidase) was added and the reaction was revealed by using diaminobenzidine. For statistical purposes, five randomly chosen visual fields were photographed at 400X using a Zeiss Axiostar plus microscope and 3,000 cells were counted for each cell experimental condition in triplicate.

Analysis of Apoptosis by Flow Cytometry

In order to detect the phosphatidylserine externalization, tumoral pituitary cells were collected with TrypLE™ Express, washed twice with PBS and suspended in 200 µL binding buffer (100 mM HEPES, pH 7.4, 140 mM NaCl, 2.5 mM CaCl2). The apoptotic analysis was performed by using Annexin V-FITC and PI, according to the manufacturer’s instructions (BD Pharmingen, USA). After incubation for 15 min at RT in darkness, 400 µL of binding buffer were added to each sample, which was then analyzed by flow cytometry (FaCScan; Ortho Diagnostic System, Raritan, NJ). Data were analyzed using the FlowJo software (Tree Star, Inc. Ashland, OR). PI and Annexin V were evaluated to distinguish late and early apoptotic events, respectively.
Supplemental Figure 1. TGFβ1 time and dose response on GH3B6 cell proliferation. Cells were treated with 2 or 4ng/ml TGFβ1 for 24 h or exposed to TGFβ1 (4ng/ml) for 7, 16 or 24 h. At the end of the experiments, cells were collected, stained with propidium iodide and analyzed for cell cycle distribution using flow cytometry. The anti-mitogenic effect of TGFβ1 was dose and time dependent, as revealed by the percentage of cells in the proliferative fraction (S + G2/M). A dose of 4ng/ml TGFβ1 with 24 h of treatment was selected for further experiments.
Supplemental Figure 2. TGFβ1 effect on GH3B6 cell apoptosis. Cells were treated with 4ng/ml TGFβ1 for 24 h and apoptosis was determined using TUNEL (A) and annexin V-FITC/PI staining (B) assays. Both methods revealed that apoptosis was not induced by the treatment with TGFβ1 for 24 h in pituitary tumoral cells. The images correspond to a representative experiment from a total of three with similar results. Bar = 20 µm.