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The Natural Mutant Receptor hGRαT556I Causes Primary Generalized Glucocorticoid Resistance through Decreased Affinity for the Ligand and Impaired Interaction with the GRIP1 Coactivator

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INTRODUCTION

Primary Generalized Glucocorticoid Resistance (PGGR) is a rare condition characterized by tissue insensitivity to glucocorticoids owing to inactivating mutations of the *hGR* gene. A new case of PGGR was reported in a patient with an adrenal incidentaloma harboring a novel heterozygous point mutation in the *hGR* gene, which resulted in threonine (T) to isoleucine (I) substitution at amino acid position 556 of the receptor (**1**).

OBJECTIVE AND HYPOTHESES

To elucidate the molecular mechanisms of action of the hGRαT556I.

METHODS

Transactivation and Transrepression Assays

CV-1 cells were transiently transfected with pRShGRα or pRShGRαT556I (0,05μg/well), pMMTV-luc (0,5μg/well) and pGL4.73[hRluc/SV40] (0,1 μg/well) (for transactivation assays) or pRSVC(p50)-NF-κB (0.0125 μg/ well), pRSVC(p65)-RelA (0.0125 μg/well), and p(IκB)3-luc (0.125 μg/well) (for transrepression assays) using lipofectamine. Forty-eight hours later, cells were exposed to increasing concentrations of dexamethasone for 24 hours. Firefly and renilla luciferase activities were determined in the cell lysates.

Dexamethasone-Binding Assays

COS-7 cells were transiently transfected with pRShGRα or pRShGRαT556I (1.5μg/well) using lipofectamine. Confluent cells were incubated with 6 different concentrations of [^{3}H]-dexamethasone at 37° C in the presence or absence of a 500-fold molar excess of nonradioactive dexamethasone for 1 hour. Dexamethasone-binding assays were performed as previously described (**2**).

Nuclear Translocation Studies

HeLa cells were transfected with pF25GFP-hGRα or pF25GFP-hGRT556I (2μg/dish) using FuGENE 6. Sixteen hours later, cells were exposed to dexamethasone (10^{-6} M) and fluorescence was detected sequentially by an inverted fluorescence microscope.

GST Pull-Down Assays

GST-fused glucocorticoid receptor-interacting protein 1 (GRIP1) (1–1462), GRIP1 (559–774), and GRIP1 (740–1217) were bacterially produced, purified and immobilized on GST beads. COS-7 cells were transiently transfected with pRShGRα and pRShGRαT556I using lipofectamine. GST pull-down assays were performed as previously described (**3**).

RESULTS

Compared with the wild-type receptor (hGRαWT), the mutant receptor hGRαT556I demonstrated a 50% reduction in its ability to transactivate the glucocorticoid-inducible MMTV promoter in response to dexamethasone (**Figure 1A**) and did not exert a dominant negative effect upon the hGRαWT. Transrepression assays showed that the hGRαT556I displayed enhanced ability to transrepress the nuclear factor (NF)-κB signaling pathway. Dexamethasone-binding assays demonstrated that the affinity of the hGRαT556I for the ligand was 50% lower than that of the hGRαWT (Kd: 21.3 ± 4.09 nM *vs.* 10.8 ± 0.99 nM, $P < 0.05$) (**Figure 1B**). There was no significant difference in the association of hGRαT556I with the glucocorticoid-response elements (GREs) following exposure to dexamethasone, indicating that the mutant receptor preserved its ability to bind to DNA. In the absence of ligand, both the hGRαWT and the hGRαT556I were predominantly localized in the cytoplasm of cells. Addition of dexamethasone resulted in slower nuclear translocation of the hGRαT556I compared with the hGRαWT (53,3 ± 1,8 min *vs.* 15,5 ± 0,46 min, $P < 0.05$) (**Figures 2A and 2B**). Finally, the hGRαT556I interacted with the GRIP1 coactivator mostly through its AF-1 domain (**Figure 3**). The above findings are likely to occur as a result of the effect of the T556I mutation on the 3D arrangement of the receptor and the electrostatic surface of the ligand-binding domain. (**Figures 4A, 4B, 4A' and 4B'**).

CONCLUSIONS

The mutant receptor hGRαT556I impairs glucocorticoid signal transduction through multiple molecular mechanisms.

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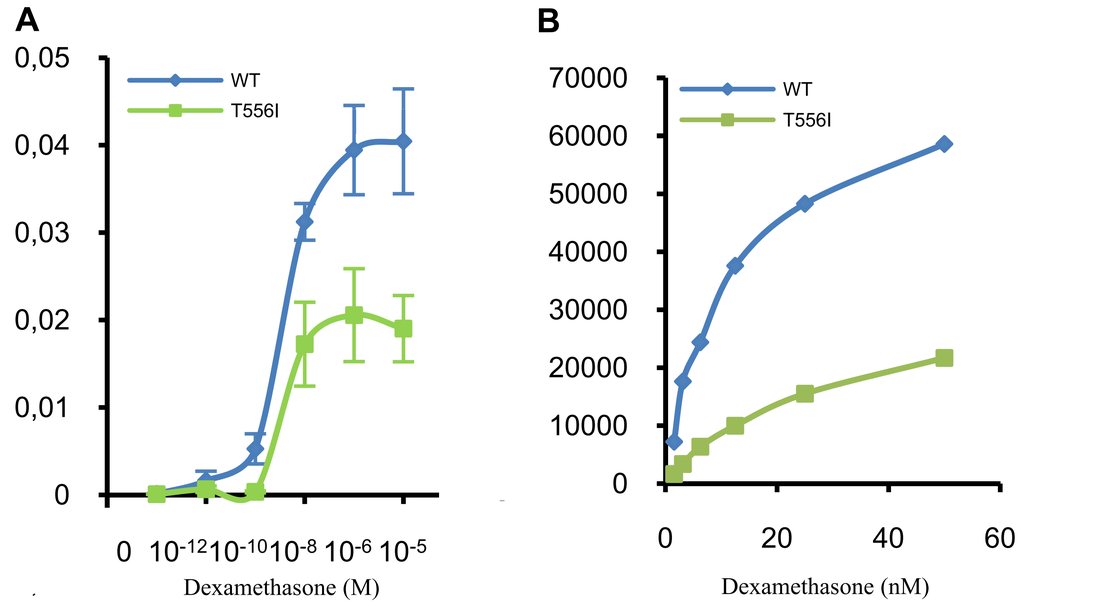

Figure 1: (A) Transcriptional activity of the mutant receptor hGRαT556I compared with the wild-type hGRα. (B) Dexamethasone-binding assays showed that the mutant receptor hGRαT556I displayed lower affinity for the ligand.

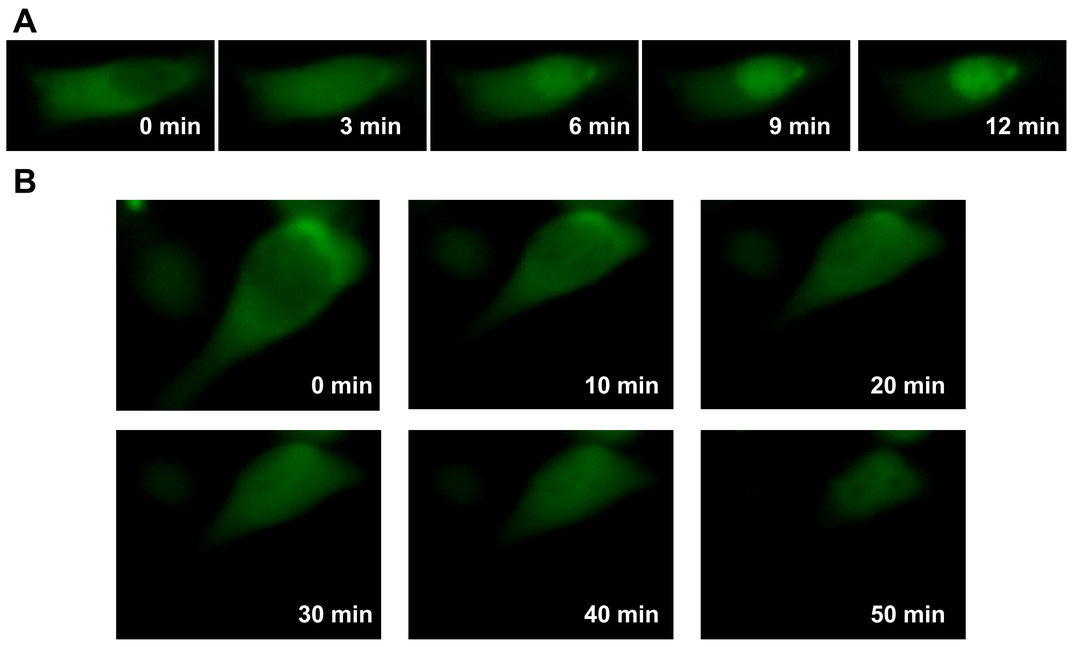

Figure 2: (A) The hGRαWT translocated to the nucleus within 12 min. (B) The mutant receptor hGRαT556I required 50 min to complete nuclear translocation.

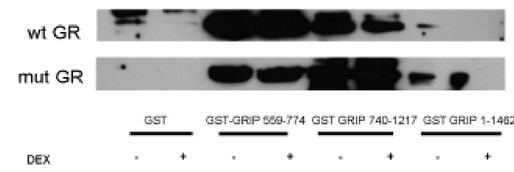

Figure 3: GST pull-down assays showed that the hGRαT556I interacted with the GRIP1 coactivator mostly through its AF-1 domain.

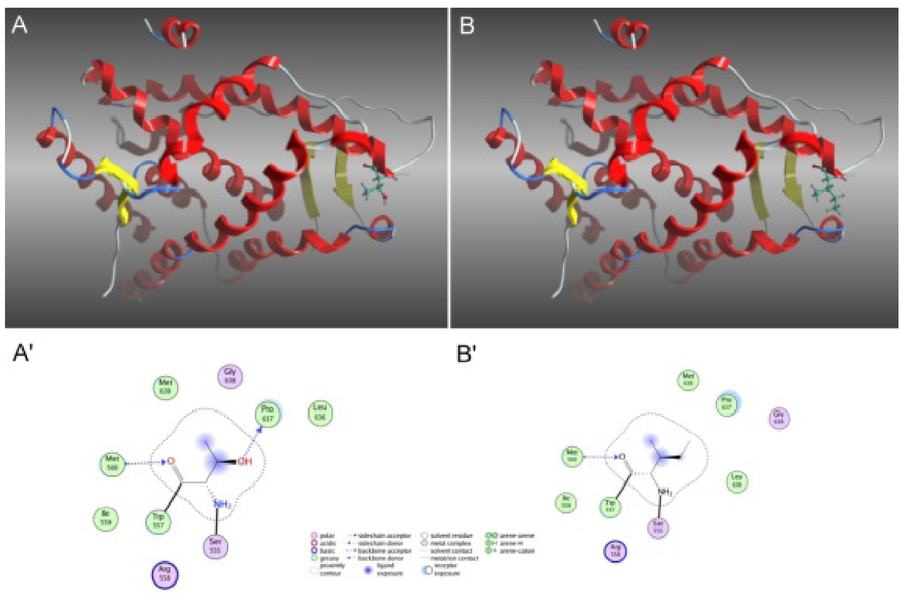

Figure 3: TheT566 wild type (A) and the T556I mutation (B) 3D structure. The interactions established by the shorter Threonine residue are completely lost when mutated to Isoleucine. More specifically the hydrogen bond between the Threonine's –OH and the P637 (A') residue is lost in the case of Isoleucine (B').

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