In vitro functional characterization of IGALS gene variants found in ALS deficient or idiopathic short stature children

Lucia Martucci, Paula Scaglia, Liliana Karabatas, Rodolfo Rey, Horacio Doméne, Sabina Doméne, Héctor Jasper

Centro de Investigaciones Endocrinológicas “Dr. César Bergadá” (CEDI E) CONICET – FEI – División de Endocrinología, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina

Background

ALS deficient (ALS-D) patients present severe IGF-I and IGFBP-3 deficiencies and variable degree of growth retardation. Heterozygous carriers for IGALS variants, ALS-D relatives or a subset of idiopathic short stature (ISS) children, have levels of IGF-I, IGFBP-3 and ALS intermediate between ALS-D and wildtype (WT) subjects. This supports that IGALS gene variants may affect ALS synthesis, secretion and/or function and could be responsible for the observed phenotype.

Objective


Methods

IGALS gene variants were introduced by site-directed mutagenesis into a plasmid containing the human IGALS cDNA. CHO cells were transiently transfected with WT-IGALS or each of the variants. Cell lysates (L) and conditioned media (CM) were analyzed by Western immunoblot. For each secreted ALS variant, new experiments were carried out and L and CM were collected at 12, 24, 36 and 48 h after transfection and immunoblotted for ALS. Besides, ALS levels in CM were measured by ELISA at 24 h post-transfection and corrected by β-galactosidase activity and total protein in L. h-TCF was performed with equal amounts of WT-ALS and secreted ALS variants by Superdex 200 size exclusion chromatography.

Results I

ALS determined by Western immunoblot of cell lysates and conditioned media from CHO cells transfected with WT-IGALS or each variant

Results II

ALS in conditioned media at 24 h post-transfection

Results III

Temporal study of WT-ALS and ALS variants in L and CM by Western immunoblot

Results IV

h-TCF of ALS-containing conditioned media incubated with rhIGFBP-3 and [125I]IGF-I

Summary and conclusions

- WT-ALS was found mostly secreted into the conditioned media at 24 hours.
- p.L213F-ALS was present in cell lysates but absent in conditioned media, suggesting an impairment on its secretion pathway.
- Secreted p.A330D-ALS levels were significantly reduced 24 h after transfection comparing to WT-ALS.
- All but one of the variants found in homozygosis or compound heterozygosis in ALS-D patients were not secreted.
- Two variants (p.E35Qfs’17 and p.A330D) found in heterozygosis in ISS patients might have a role in the pathogenesis of their short stature.

Supported by PICT 2010 Nº 1918 (ANPCYT) and SANDOZ International GmbH, Business Unit Biopharmaceuticals.

The authors have nothing to disclose.

DOI: 10.3252/pso.eu.54espe.2015