Effects of Glypican-4 Protein on INS1E Cell Viability and Insulin Signaling

J. Buhl1, A. Garten1,2, S. Richter1, M. Vogel3, J. Kratzsch4, W. Kiess1,3, M. Penke1

1 Center for Pediatric Research Leipzig, University Hospital for Children and Adolescents, Leipzig University, Germany
2 Institute of Metabolism and Systems Research, University of Birmingham, United Kingdom
3 LIFE Leipzig Research Center for Civilization Diseases, Leipzig University, Germany
4 Institute for Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, Leipzig University, Germany

BACKGROUND

Glypican-4 is a heparan sulfate proteoglycan, released from human and rodent adipose tissue and shown to regulate insulin signaling through direct interaction with the insulin receptor, enhancing cell viability, and insulin signaling. Because of its positive effect on insulin sensitivity, glypican-4 might play a role in the development of obesity, insulin resistance, and type 2 diabetes (T2D).1,2 Glypican-4 serum levels are associated with obesity and insulin resistance.2-3 Several studies revealed a positive correlation between glypican-4 and obesity-related parameters, e.g., weight, BMI, WHR, body fat content.3,4,5

Glypican-4 is an interesting new adipokine and might link obesity and T2D. Because of the fact that studies concerning this protein, specifically measuring circulating levels in lean and obese children, are rare, we found it interesting to investigate this heparan sulfate proteoglycan. We hypothesized: Glypican-4 plays an essential role in cell function and want to answer the following questions: 1. Do INS1E cells express glypican-4? 2. Does extracellular glypican-4 influence its cell function? 3. Does glypican-4 link obesity to T2D?

RESULTS

Figure 1. INS1E cells express glypican-4.

A. Western blot detection of glypican-4 protein in INS1E cells and different rat tissues. Positive control: K562 cell lysate (A), rat adipose tissue (B), loading control: GAPDH (A, B), β-Actin (B). C, D: Western blot detection of Glypican-4 mRNA in INS1E cells and different rat tissues. Each sample was measured in triplicates (n=3 independent experiments). As control rat adipose tissue (rat AD) was used and arbitrarily set 1. Data are shown as mean ± SEM, normalized to rat Hprt1 and rat 18S and analyzed using the AAO method.

Figure 2. Recombinant glypican-4 has no effect on INS1E cell viability and insulin signaling.

A, B, C: Determination of INS1E cells with recombinant rat glypican-4 for 10 days. Western blot detection of pAKT/AKT, pERK/ERK, and GAPDH protein. One representative Western blot is shown. Each sample was measured in triplicates (n=3 independent experiment) using ImageJ for densitometric analysis of the Western Blots. Data are shown as mean ± SEM, normalized to total protein amount. D: WST-1 assay measurement of INS1E cell viability. Each sample was measured with WST1-Assay in quintuplicates (n=2 independent experiments) after 6h, 24h and 72h of incubation with recombinant rat glypican-4 protein. Stimulants were dissolved in 1% (v/v) Serum. Media. Data are shown as mean ± SEM. Absorbance correlates positively with cell viability. The average over replicates based on blank corrected absorbance is given.

Figure 3. Validation of two different human Glypican-4 EUSA kits.

A: Western blot detection of inter-assay variation of glypican-4 protein in human glypican-4 (EUSA kit) and mouse glypican-4 (EUSA kit) in control sera and sera of obese, diabetic patient. We did not detect glypican-4 in any form (serum, recombinant, recombinant spiked in serum) with the other EUSA kit (Sclaryco Antibodies). B Validation results of Cloud Clone Corp. human glypican-4 EUSA kit. We could not detect any signal when using recombinant human glypican-4 expressed in E. coli in spiking experiments. Since a potential reason is incorrect folding because of the heterologous expression, we expressed glypican-4 in a mammalian system (HEK293) for use in validation experiments. C: Immunoprecipitation and Western blot detection of human recombinant glypican-4 protein expressed in HEK293 cells.

CONCLUSION

1. INS1E cells express both glypican-4 protein and mRNA. The expression of glypican-4 mRNA is high in the metabolic organs kidney, muscle, pancreas, and liver. In contrast, glypican-4 protein was found to be abundant in lungs, kidney, heart, and pancreas.

2. Exogenous glypican-4 seems to have a negative effect on cell viability, enhancing cell viability, and insulin signaling were not significantly influenced after stimulation with recombinant glypican-4 protein.

3. To measure glypican-4 concentration in serum samples of the LIFE Child cohort, we validated two different human Glypican-4 EUSA kits. One of them has not measured any glypican-4. Validation results of the other kit are pending.