A Novel Interaction Between Synaptojanin-1 and ZO-1 Indicates Clathrin-Mediated Turnover at the Slit-Diaphragm

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Background

The importance of clathrin-mediated endocytosis (CME) for podocyte health has long been established. Deletion of Synaptojanin 1 (Synj1), which is involved in the uncoating of clathrin-coated vesicles (CCV), resulted in severe albuminuria and foot process effacement in mice. The aim of this study is to further understand the underlying mechanism and role of Synj1 at the slit-diaphragm (SD).

Methods

We created a mouse monoclonal antibody specific for the Synj1 isoform expressed in podocytes. Mass Spectrometry analysis following immunoprecipitation (IP) of podocyte lysate revealed tight junction protein, Zonula occludens 1 (ZO-1) as a binding partner. To further identify the binding domains, we transfected Cos7 cells with various tagged truncated ZO-1 and Synj1 plasmids and performed IP. We analyzed through live imaging, the temporal and spatial relationship between ZO-1 and clathrin in isolated control podocytes. Immunofluorescence (IF) staining with ZO-1 and clathrin light-chain antibodies on wild-type and *Synj1* KO mouse podocytes was performed to determine ZO-1 localization.

Results

The association of Synj1 with ZO-1 was confirmed by IP in Cos7 cells overexpressing Synj1 and ZO-1. Binding between the SH3 domain of ZO-1 and proline-rich domains of Synj1 was observed. IF staining of wild-type mouse podocytes demonstrated ZO-1 co-localized with

clathrin-coated pits. Live cell imaging further revealed CME of ZO-1. However, Synj1 KO podocytes displayed ZO-1 mislocalization in the cytosol when compared to wild-type.

Conclusion

We identified a novel binding partner of Synj1, the slit-diaphragm protein ZO-1, which indicates clathrin-mediated turnover of ZO-1 similar to Nephrin likely occurs. IF images and live cell imaging further corroborated these findings. Furthermore, inhibiting uncoating of CCV in Synj1 KO podocytes resulted in mislocalization of ZO-1. These findings illustrate the importance of clathrin-mediated recycling of slit-diaphragm proteins during podocyte health and disease.

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