Freie Universität Berlin

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The role of ARFRP1-mediated *trans*-Golgi scaffolds on retromer function

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Project A13

Collaboration Project/s (if applicable):

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Abstract:

In Project A13 we study the role of the small GTPase ARFRP1 on the formation of *trans*-Golgi residing scaffolds that influence secretion and recycling processes. In the current funding period we could show that the specific deletion of *Arfrp1* in mature fat depots of mice led to mistargeting of the insulin receptor accompanied by reduced insulin sensitivity of the adipose tissue [A]. In collaboration with Prof. O. Daumke (A12) we screened for new putative ARFRP1 interacting proteins and identified 23 potential candidates with annotated Golgi function. One candidate, VPS35 that is part of the mammalian retromer complex, showed the same tendency than ARFRP1 towards altered insulin receptor recycling when knocked down by specific siRNA in 3T3-L1 adipocytes (Fig. 1A). However, a direct interaction of both proteins could not be verified, as analyzed by SIM-technique together with Z02. Another putative ARFRP1 interacting protein is the *trans*-Golgi scaffolding protein GOPC that shows strong co-localization together with ARFRP1 and, Golgin245 after immune labelling and confocal microscopy (Fig. 1B, C).
The requested funding will be used to support a PhD student who will start his/her work before the new funding period will start. The candidate will analyze the supposed interaction of ARFRP1 and GOPC, VPS35 and other retromer proteins by co-expression in HeLa cells followed by Co-IP experiments. He/she will perform Nano-trap experiments to clarify which protein directly interacts with ARFRP1. According to the strong co-localization of ARFRP1, Golgin245 and GOPC (Fig 1.B, C) we believe that GOPC is the most likely direct interaction partner of ARFRP1. Therefore, truncated versions of GOPC will be used to map the interacting domains. Each single putative binding partner will be suppressed by siRNA in 3T3-L1 adipocytes to study insulin receptor localization. Functional assays, e.g. biotinylation of the insulin receptor, will be performed after siRNA mediated knockdown of the indicated candidates. The putative binding partner will also be suppressed in HeLa cells followed by transferrin uptake and recycling assays in order to evaluate their role on endosomal mediated exocytosis because suppression of ARFRP1 markedly impairs this process [A]. Furthermore, the collaboration with Z02 will be extended to precisely analyze the subcellular localization of ARFRP1, Golgin245, GOPC and VPS35 together.

Requested funding: One PhD student stipend for 12 months starting 07/2018.

Publication/s:

[A] Rödiger M, Werno MW, Wilhelmi I, Baumeier C, Hesse D, Wettschureck N, Offermanns S, Song K, Krauß M, Schürmann A. Adiponectin release and insulin receptor targeting share trans-Golgi-dependent endosomal trafficking routes. Mol. Metab. 12-8778 (17) 2017

Other related publications:

Werno MW, Wilhelmi I, Kuropka B, Schuehle S, Freund C, Schürmann A. Trans-Golgi associated GTPase ARFRP1 is involved in lipid droplet assembly and triglyceride release from intracellular storage of intestinal Caco-2 cells. Biochem Biophys Acta under review

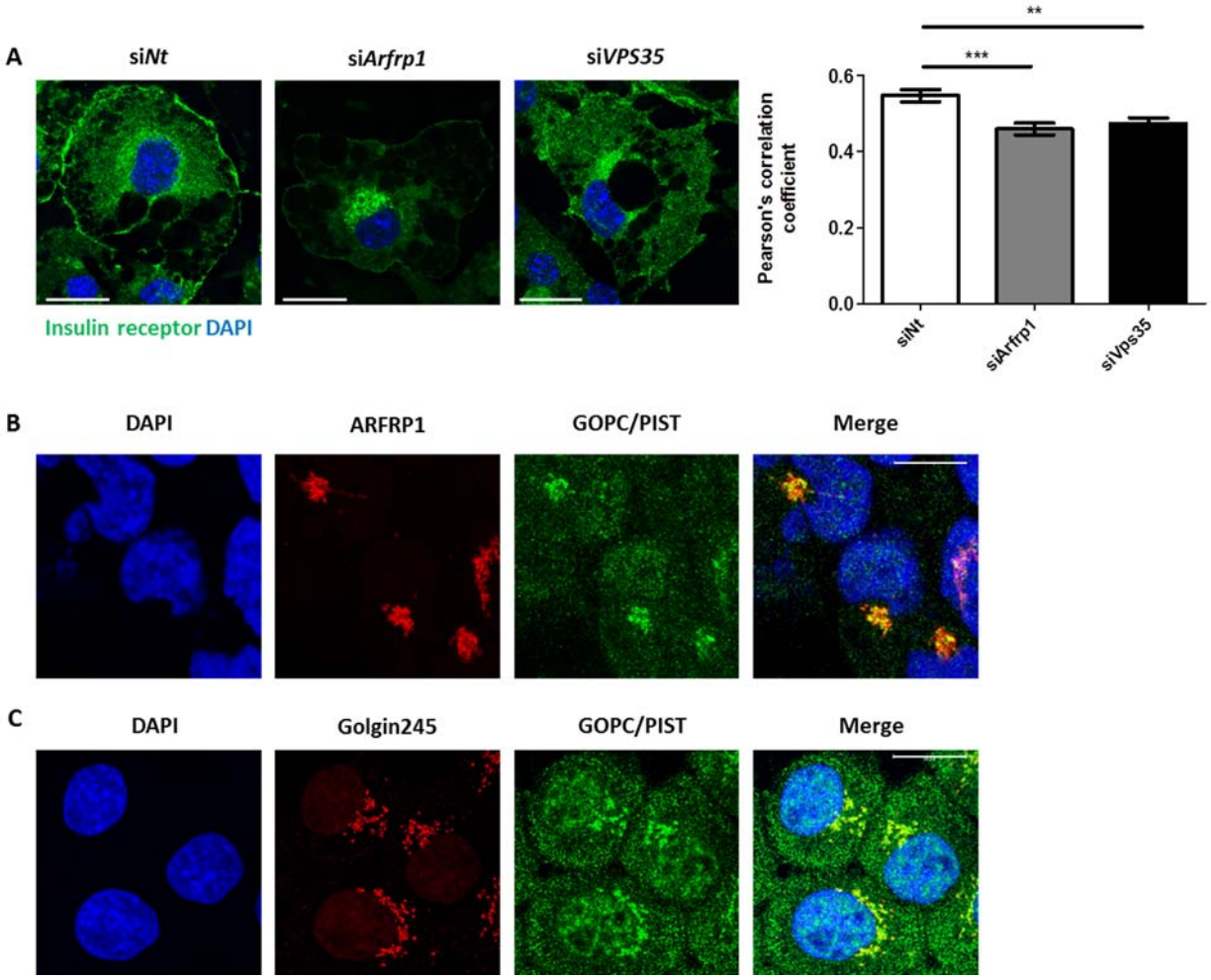

Figure 1: Characterization of new putative ARFRP1 interacting proteins. A: Analysis of insulin receptor sorting in 3T3-L1 adipocytes. siRNA treated 3T3-L1 cells were serum starved for 4 hours before staining of insulin receptor (green) and nuclei by DAPI (blue), scale bar represents 20 µm. Quantification of insulin receptor at the plasma membrane shown as mean +/- SEM from 4 independent experiments. **B:** GOPC/PIST colocalizes with ARFRP1 in HeLa cells. HeLa cells were stained for ARFRP1 (red), GOPC/PIST (green) and nuclei by DAPI (blue). Scale bar represents 20 µm. **C:** GOPC/PIST colocalizes with Golgin245 in HeLa cells. HeLa cells were stained for Golgin245 (red), GOPC/PIST (green) and nuclei by DAPI (blue). Scale bar represents 20 µm.