Supplementary Material

Expression and Localization of AβPP in SH-SY5Y Cells Depends on Differentiation State

Supplementary Methods

Western blot analysis

First, SH-SY5Y cells were lysed using an ice-cold radioimmunoprecipitation assay buffer (RIPA; pH 8.0; 150 mM NaCl, 0.1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS 50 mM TRIS-Base) supplemented with inhibitors of proteases and phosphatases on ice for 40 min. After blocking with 5% skim milk at room temperature (RT) for 1 h, PVDF membranes with proteins separated on 12% SDS-PAGE were incubated with primary antibodies at 4°C overnight. Next, membranes washed with TBS containing 0.1% Tween-20 were incubated with secondary antibodies conjugated with horseradish peroxidase at room temperature for 1 h. The signal was developed with Clarity™ Western ECL Substrate (BioRad, 1705060) and imaged on the gel documentation system (BioRad). The protein expression was quantified using ImageJ [1,2] and normalized to the GAPDH signal.

Quantitative real-time PCR

Total RNA was isolated from the $25x10^4$ SH-SY5Y cells using 1ml of TRI reagent (Sigma-Aldrich, T9424). The RNA was treated with DNAse I (Sigma-Aldrich, AMPD1) at 37°C for 30 min, precipitated with 8 M LiCl (Sigma-Aldrich, L7026) followed by incubation overnight at −20°C and centrifugation for 30 min at 16 000 × g. After washing twice with 1 ml of 80% ethanol and centrifugation for 30 min at full speed, the total RNA was resuspended in 1xTE buffer and evaluated using Fragment Analyzer (Standard Sensitivity RNA analysis kit; AATI, DNF-471). 500 ng of total RNA was reverse transcribed with TATAA GrandScript cDNA SuperMix (TATAA

Biocenter, AS103b). Real-time PCR analysis (for primers, see Supplementary Table 1) was performed on the C-1000 and CFX384 (BioRad) using Sybr GrandMaster Mix (TATAA Biocenter, TA01-625). Relative AβPP and BACE1 expression levels were calculated using the $2^{-\Delta\Delta CT}$ method and normalized to the reference GAPDH gene. Each experiment was performed in technical triplicates.

Super-resolution localization microscopy

Image acquisition for direct stochastic optical reconstruction microscopy (dSTORM) was performed on the microscope setup described above under highly inclined and laminated optical sheet illumination (HILO). Before recording, all activated fluorophores were bleached by intense 643 nm illumination. The acquisition was performed in the reducing STORM imaging buffer (pH 8.0; 50 mM Cysteamine, 50 mM TRIS, 10 mM NaCl, 1 688 AU glucose oxidase, 14 040 AU catalase, 8.5% glucose) with 50 ms frame rate interleaved with 1.5 ms pulses of 405 nm activation laser. The intensity of the activation light was increased during the acquisition to achieve the density of one emitter per μm2 in each frame. Typically, 20, 000 frames were collected. Thunderstorm plugin for ImageJ [3] was used for the analysis and reconstruction of dSTORM images.

Colocalization analysis

MitoTracker, mApple-Golgi-7, and AβPP channels were deconvolved using 2D iterative deconvolution from Parallel iterative deconvolution package [4] using WPL method with default settings. PSF for each channel was determined by the measurement of TetraSpecks beads (0.1 μm, Molecular Probes). The background was subtracted, and ROI was selected based on the cell

outlines in the brightfield image. JACoP plugin for ImageJ [5,6] was used to calculate Manders' M1 coefficient based on automatic threshold.

Supplementary Table 1 Primers used for real-time PCR.

Primer name	Sequence	Source
hAβPP_fwd	AGACTATGCTGATGGCGGTGAAG	[7]
hAβPP_rev	CAATGCTGGTTGTTCTCTCTGTGG	[7]
hBACE1_fwd	ACGTGGAGATGACCGTGGG	This work
hBACE1_rev	GAGGTCCCGGTATGTGCTG	This work
GAPDH_fwd	TGCACCACCAACTGCTTAGC	This work
GAPDH_rev	GGCATGGACTGTGGTCATGAG	This work

REFERENCES

[1] Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, Arena ET, Eliceiri KW (2017) ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics* **18**, 529.

[2] Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B (2012) Fiji: an open-source platform for biological-image analysis. *Nat Methods* **9**, 676–682.

[3] Ovesný M, Křížek P, Borkovec J, Švindrych Z, Hagen GM (2014) ThunderSTORM: a comprehensive ImageJ plug-in for PALM and STORM data analysis and super-resolution imaging. *Bioinformatics* **30**, 2389–2390.

[4] ImageJ, Parallel Iterative Deconvolution 2D and 3D (ImageJ).

[5] Bolte S, Cordelières FP (2006) A guided tour into subcellular colocalization analysis in light microscopy. *J Microsc* **224**, 213–232.

[6] Cordelieres FP, Bolte S (2008) JACoP v2. 0: improving the user experience with co-localization studies. In *Proceedings of the 2nd ImageJ user and developer conference*, pp.

6–7.

[7] De Chiara G, Marcocci ME, Civitelli L, Argnani R, Piacentini R, Ripoli C, Manservigi R, Grassi C, Garaci E, Palamara AT (2010) APP processing induced by herpes simplex virus type 1 (HSV-1) yields several APP fragments in human and rat neuronal cells. *PLoS One* **5**, e13989.