Increased ribosomal protein levels and protein synthesis in the striatal synaptosome of Shank3-overexpressing transgenic mice

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Main text
Shank3 protein is a core organizer of the macromolecular complex in the postsynaptic density (PSD) of neuronal excitatory synapses [1]. Consistent with its critical roles in proper synaptic development and function, variants of the SHANK3 gene have been causally associated with numerous synaptopathies [2]. Shank3 interacts with many other synaptic proteins through its multiple protein–protein interaction domains, thereby regulating their synaptic localization and stability [3]. This “scaffolding” function is a well-established mechanism underlying the organization of the PSD complex by Shank3. However, considering the highly dynamic regulation of synaptic proteins, including local synthesis and turnover [4], there can be

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additional, yet unexplored, mechanisms through which Shank3 orchestrates the postsynaptic proteome. By applying a quantitative proteomic analysis, we recently identified several differentially expressed (63 up-regulated/73 down-regulated) proteins in the striatal synaptosome of Shank3 TG mice compared with wild-type (WT) mice [5]. Unexpectedly, we found that six ribosomal proteins (RPs) were included in the up-regulated proteins of the TG striatal synaptosome (Fig. 1a). Consistently, bioinformatic analyses revealed that several ribosome-related terms were significantly represented by the up-regulated proteins (Additional file 1: Figs. S1 and S2). Furthermore, Western blot analysis validated the increased levels of RPLP1 and RPL36A in synaptosome of the TG striatum compared with the WT striatum (Fig. 1b). Notably, in whole lysate, those protein levels were comparable between the TG and WT striata, suggesting that an increase in RP levels was specific to synaptosome. Based on the above results, we measured the efficiency of global protein synthesis or mRNA translation in WT and TG striata, by using a puromycin incorporation assay (Additional File 1: materials and methods). We used acute striatal slices from WT and TG mice and confirmed that proteins in both whole lysate and synaptosome could be labeled by incubating the slices with puromycin (Additional File 1: Fig. S3). Labeling was suppressed by pretreatment with the protein synthesis inhibitor, cycloheximide, suggesting that puromycin signals represent nascent polypeptides. When we measured the intensity of puromycin labeling, it was significantly increased in synaptosome, but not in whole lysate, of the TG striatum compared with the WT striatum (Fig. 1c). This result suggests that, similar to the increase in RP levels, protein synthesis was enhanced only in synaptosome of the TG striatum.

The mechanistic target of rapamycin (mTOR) and mitogen-activated protein kinase (MAPK)/ERK pathways are key regulators of synaptic protein synthesis [6]. We previously showed decreased mTOR complex 1 (mTORC1) activity in whole lysate of the TG striatum compared with the WT striatum [7, 8]. However, since increased RP levels and protein synthesis in the TG striatum were observed specifically in synaptosome, we measured the activities of mTORC1 and ERK in the striatal synaptosome of WT and TG mice. Unlike in whole lysate [7], mTORC1 activity was normal, but ERK activity was significantly increased in the TG striatal synaptosome (Fig. 1d). The total levels of mTOR and ERK proteins were comparable between the WT and TG striata.

Intriguingly, when we compared the list of 63 up-regulated proteins in the TG striatal synaptosome [5] with that of the recently reported comprehensive Shank3 interactome (793 proteins) [9], only eight proteins, including Shank3 itself, were shared by both lists (Additional File 2: Table S1). This unexpectedly low percentage of Shank3-interacting proteins in the up-regulated proteome (12.7%) suggests that interaction-mediated recruitment by overexpressed Shank3 (i.e., the scaffolding function of Shank3) may only partially contribute to the proteomic change in the TG striatal synaptosome. Therefore, we investigated whether increased protein synthesis may be associated with proteomic change in the TG striatal synaptosome. To examine this, we performed sucrose density-gradient fractionation with the striatal synaptosome of WT and TG mice and then purified RNAs from whole synaptosomal (referred to as “input”) or only polysome-enriched (“polysome”) fractions (Fig. 1e). We validated the relative enrichment of mRNAs in the polysome fractions by measuring the amounts of two well-known synaptic mRNAs (CaMKIIα andDlg4) [10] (Fig. 1f). The purified synaptosomal input
and polysome RNAs from three pairs of WT and TG striatal samples were further processed for next-generation RNA-sequencing (RNA-seq) (Additional File 2: Table S2).

We previously performed whole tissue, as opposed to synaptosome-enriched, RNA-seq on the WT and TG striata [7]. Therefore, using the obtained multi-omics datasets, we could compare correlations between proteomic change and three different transcriptomic changes in the TG striatum (Fig. 1g). Using proteomic change as a standard, we calculated its correlations with each of the three RNA-seq datasets by matching their fold-change values for each protein. Notably, as we narrowed down the list of proteins, from the top 700 (350 up-regulated/350 down-regulated) to the top 100, based on their fold-change values, mRNA level change in polysome, but not in whole tissue and input,
showed a gradual increase in the correlation coefficient to the proteomic change (Fig. 1h–j). In addition, with any group of protein lists, polysome showed a markedly higher correlation coefficient than the whole tissue and input coefficients. Moreover, when we separately analyzed the top 50 up-regulated and 50 down-regulated proteins, only the up-regulated proteins showed a further increase in correlation coefficient with respect to the polysome data (Fig. 1k). These results suggest a positive correlation between polysome-associated transcriptomic and up-regulated proteomic changes in the TG striatal synaptosome.

Here, we showed increased RP levels and protein synthesis in the striatal synaptosome of Shank3-overexpressing mice. Elevated ERK activity, which directly interacts with Shank3 [11], may mediate the process [6, 12]. Based on our comparative analyses, we cautiously speculate that increased protein synthesis from the synaptic polysome-associated transcripts in the TG striatum may contribute to the remodeling of its postsynaptic proteome (Fig. 1l). The correlation between them might be underestimated because our proteomic data is a “snapshot” which is a net outcome of protein synthesis and degradation. Therefore, direct identification of the locally synthesized proteome [13] in the TG striatal synaptosome will be an important direction for future studies.

Among the up-regulated proteins in the TG striatum, dopamine D1 receptor (DRD1) and its downstream target, dopamine and cAMP regulated phosphoprotein 32 kDa (DARPP-32), showed up-regulated polysome-associated mRNA levels in the TG striatal synaptosome (Additional File 2: Table S3). Considering mania-like behaviors, such as hyperlocomotion and amphetamine hypersensitivity, observed in Shank3 TG mice [3, 14], increased local synthesis of DRD1 and DARPP-32, if validated, may possibly contribute to the synaptic and behavioral changes in TG mice.

In conclusion, our study provides evidence suggesting the convergence of synaptic scaffolds and protein synthesis, where abnormalities are considered major pathogenic mechanisms underlying numerous synaptopathies [15, 16].

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s13041-021-00756-z.

Additional file 1: Fig. S1 Gene ontology analysis for the up-regulated (A) and down-regulated proteins (B) in the striatal synaptosome of Shank3 TG mice. Fig. S2 Gene set enrichment analysis (GSEA) for the proteomic change in the striatal synaptosome of Shank3 TG mice. Fig. S3 Puromycin (Puro) labeling of nascent polypeptides in acute slices of the mouse striatum. CHX, cycloheximide. Materials and methods

Additional file 2: Table S1. List of 63 up-regulated proteins in the striatal synaptosome of Shank3 TG mice. Table S2. Summary of RNA-seq mapping results. Table S3. List of proteins identified from the quantitative proteomic analysis in the striatal synaptosome of Shank3 TG mice, and their fold changes values for each data set are shown.

Additional file 3. All raw images for entire membranes of Western blotting.

Additional file 4. All raw numerical data for image quantification.

Abbreviations
DAHP-32: Dopamine and cAMP regulated phosphoprotein 32 kDa; DRD1: Dopamine D1 receptor; ERK: Extracellular signal-regulated kinase; MAPK: Mitogen-activated protein kinase; mTOR: Mechanistic target of rapamycin; PSD: Postsynaptic density; RNA-seq: RNA-sequencing; RP: Ribosomal protein; Shank3: SH3 and multiple ankyrin repeat domains 3; TG: Transgenic; WT: Wild-type.

Acknowledgements
We thank the Laboratory Animal Research Center at the Korea University College of Medicine for animal care and support.

Authors’ contributions
CJ, YL, KJ, JP YZ, HRK, RM, HS, YK, and KH designed and performed the experiments. HK, HJ, JYK, YKK, and KH analyzed and interpreted the data. KH wrote the paper. All authors have read and approved the manuscript.

Funding
This work was supported by the National Research Foundation of Korea (NRF) grants funded by the Korea Government Ministry of Science and ICT (NRF-2018R1C1B6001235 and NRF-2018M3C7A1024603 to KH), by the Korea University Graduate School Junior Fellow Research Grant (to CJ), and by the KBSI Grant (C060100 to JYK).

Availability of data and materials
The datasets used and analyzed in the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The Shank3 TG mice were bred and maintained in a C57BL/6J background according to the Korea University College of Medicine Research Requirements. All experimental procedures were approved by the Committee on Animal Research of the Korea University College of Medicine (KOREA-2018-0003).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 22 December 2020 Accepted: 19 February 2021
Published online: 23 February 2021
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