The Novel Involvement of PTSD-Associated FKBP5 in Protein Networks and Signaling Pathways

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Abstract

The association between FK506-binding protein 5 (FKBP5), a negative-feedback regulator of the glucocorticoid receptor, and PTSD, a debilitating stress-related disorder, has been extensively documented. However, the underlying biology of this connection remains largely elusive. Our study aimed to investigate the FKBP5-involved connection at the protein-protein interaction and signal levels in vitro.
We employed mass spectrometry (MS/MS) and analyzed Gene Ontology (GO) term enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways through the Search Tool for the Retrieval of Interacting Genes database (STRING), ShinyGO 0.77. We examined the FKBP5 profiling of the dexamethasone (Dex)-induced protein-protein interaction network and signaling pathways in an in vitro pharmacological model using HS-SY5Y cells. For co-immunoprecipitation (CO-IP), we utilized a monoclonal antibody directed at FKBP5 and its interacting proteins in the cells' extracted proteins. Subsequently, we trypsin-digested immunoprecipitated and cross-linked proteins on beads, followed by liquid chromatography–mass spectrometry (LC-MS/MS) analysis of the peptides. We measured the relative expression of proteins between treated and untreated samples, identifying distinct protein-protein interaction networks. Applying a fold change cutoff value of 2.0, we identified time-dependent up- and down-regulation of Dex-induced FKBP5-associated proteins. The up-regulation of FKBP5-associated proteins induced by Dex was prominent in the mitochondria, while the down-regulation was notable in the cytosol. Our findings suggest a complex relationship between glucocorticoid signaling and FKBP5, with potential implications as biomarkers. Dynamic FKBP5 redistribution might be an essential event in cell signaling, linking glucocorticoid receptor function with the mitochondria, cytosol, and nucleus in PTSD or under stress hormone exposure.

Materials and Methods

In this study, SH-SY5Y human neuroblastoma cells were cultured in Advanced DMEM with 10% FBS at 37°C and 5% CO2. Cells were exposed to 1μM dexamethasone (DEX) or a control vehicle for 6, 24, and 72 hours.
Subcellular fractions were isolated using the Thermo Scientific Subcellular Protein Fractionation Kit for Cultured Cells (#78840). Initially, cells were harvested, pelleted, and resuspended in cold PBS. Cytosolic fractions were extracted using cytoplasmic extraction buffer (CEB) with protease and phosphatase inhibitors, followed by centrifugation. Membrane fractions were isolated from the remaining pellet using membrane extraction buffer (MEB) with protease inhibitors. Vortexing and centrifugation yielded the membrane extracts. Nuclear fractions were prepared by treating the pellet with nuclear extraction buffer (NEB) containing protease inhibitors, $CaCl_2$, and Micrococcal Nuclease. After appropriate incubation and centrifugation, nuclear extracts were obtained. Mitochondrial fractions were isolated with the Thermo Scientific Mitochondria Isolation Kit for Cultured Cells (#89874). Protein concentrations were determined with the Thermo Scientific Pierce BCA protein assay kit (#23227). Co-immunoprecipitation (co-IP) was performed using Thermo Scientific Pierce MS-Compatible Magnetic IP Kits (#90409), with 5μg of FKBP5 antibody and Protein A/G magnetic beads. These methods allow the comprehensive examination of cellular responses to DEX treatment and protein-protein interactions (PPI) in subcellular fractions.

PPI network construction, cluster analysis, and key protein identification: To predict protein-protein interactions and analyze the interaction relationships between proteins in our study, we constructed a PPI network using the online STRING database (https://string-db.org/) with a cutoff of interacting confidence scores of >0.4 designated as the. The results were presented as visualized figures of the interactions among the dysregulated chemokines. **Functional enrichment analysis:** To identify the role of differentially expressed proteins in the Dex-treated cells, pathways of KEGG, GO biological process, and molecular function were performed using the ShinyGo 0.77. The adjusted $P < 0.05$ was considered statistically significant.

Results

Fig 1.

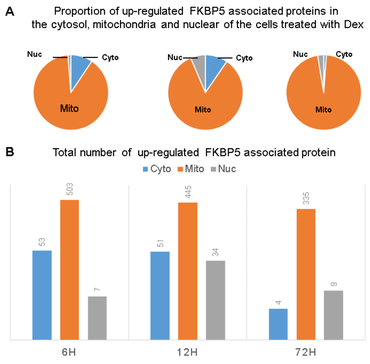


Fig 2.

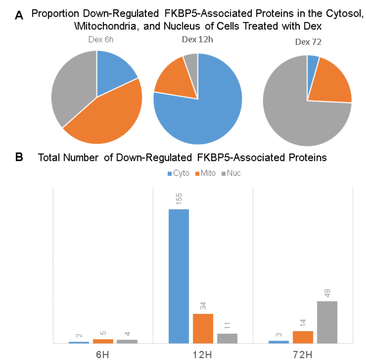


Fig 3.

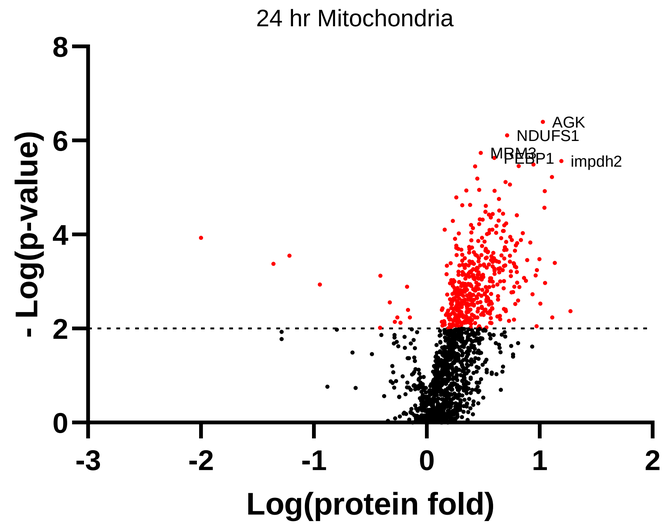


Fig. 4

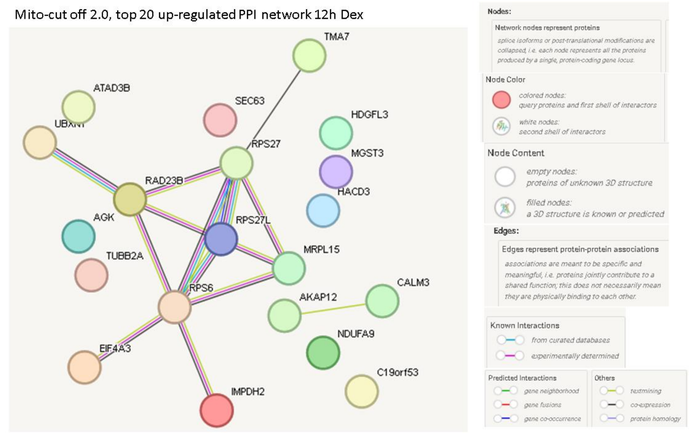
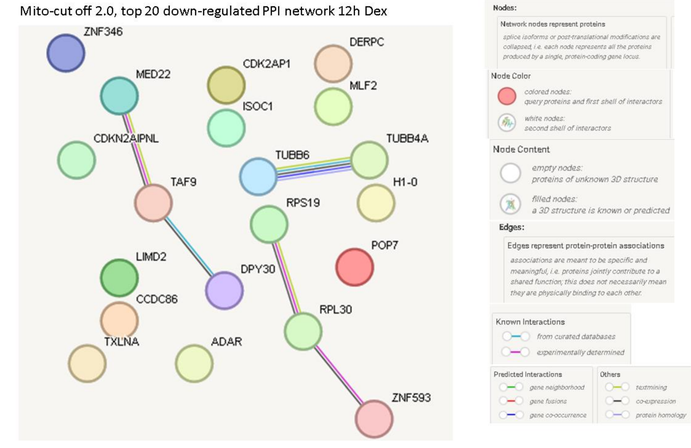


Fig. 5



Conclusion

1. Time-dependent up- and down-regulation of Dex-induced FKBP5-associated proteins was observed.
2. The up-regulation of FKBP5-associated proteins induced by Dex was prominent in the mitochondria, while the down-regulation was notable in the cytosol.
3. These findings suggest a complex relationship between glucocorticoid signaling and FKBP5, with potential implications as biomarkers. Comprehensive in vivo studies are warranted to further explore these observations.