Protein interactions of DPP3 and their putative impact on NRF2-KEAP1 signaling

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INTRO/BACKGROUND

DPP3, a zinc metallopeptidase, is an essential component of the human proteome with diverse roles in protein turnover, blood pressure regulation, and pain modulation. Recent evidence suggests its involvement in the **NRF2-KEAP1** oxidative stress response pathway through the interaction with **KEAP1**. However, the complete scope of DPP3's protein interactions remain largely unexplored.

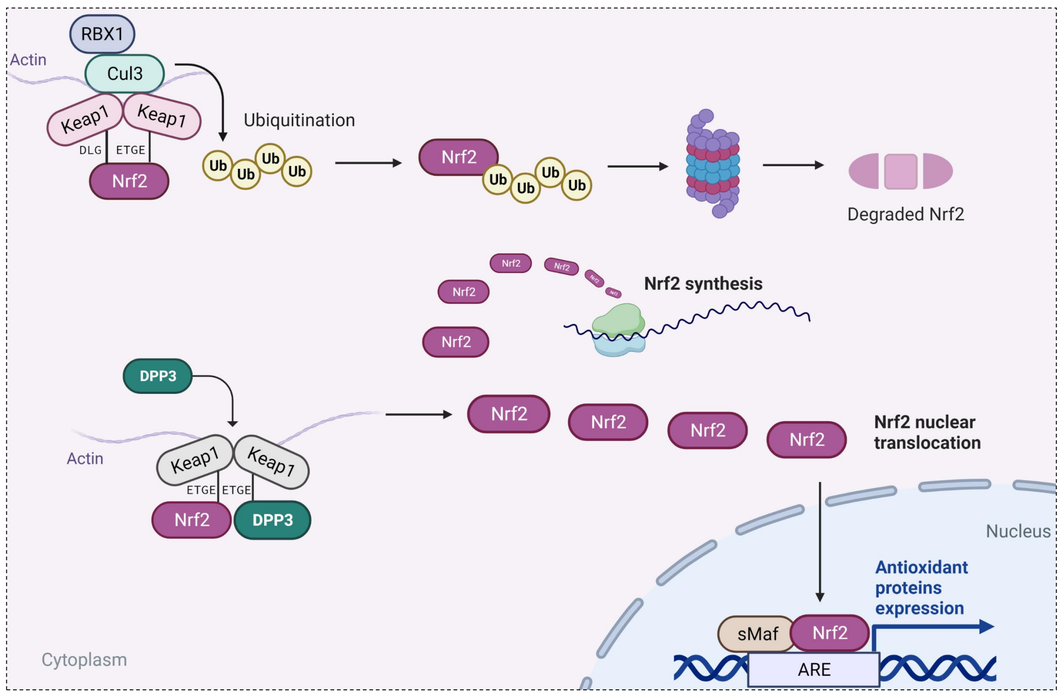

Figure 1. Involvement of DPP3 in the KEAP1-NRF2/ARE signaling pathway. Schematic representation of KEAP1-NRF2 signaling pathway including the moonlightning activity of DPP3 as an activator of the expression of genes encoding cytoprotective enzymes involved in the oxidative stress response.

METHODS

To identify novel protein interactors of DPP3, SILAC-MS approach was employed using HEK293T cells stably expressing HA-DPP3 as the bait. This technique allows for the detection and quantification of proteins based on isotopic labeling. Four independent experiments were conducted, and mass spectrometry analysis was utilized to identify potential interactors. To validate the selected interactions, co-immunoprecipitation (co-IP) experiments were conducted utilizing endogenous proteins. Binding was analysed by western blotting.

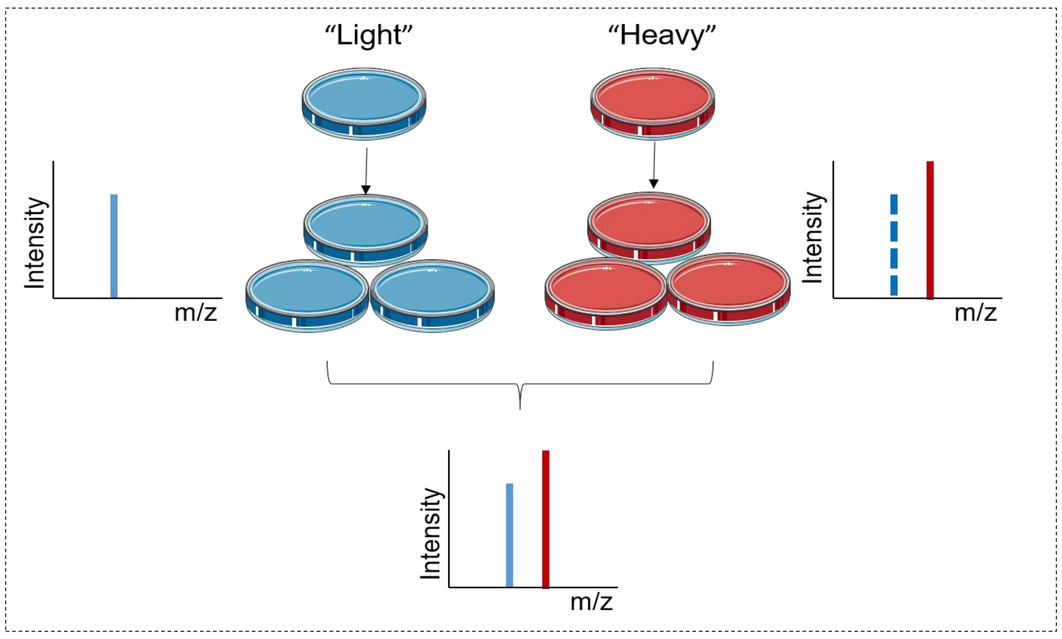

Figure 2. Experimental strategy for determining DPP3 interactors. SILAC–based quantitative MS approach for real-time recording protein-protein interactions. (A) Co-immunoprecipitation using magnetic beads for detecting endogenous DPP3 interactors. (B)

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RESULTS

Potential **interactions** are crucial for unraveling the intricate **mechanisms underlying NRF2-KEAP1 signaling** and its implications for noncommunicable diseases **(NCDs)**

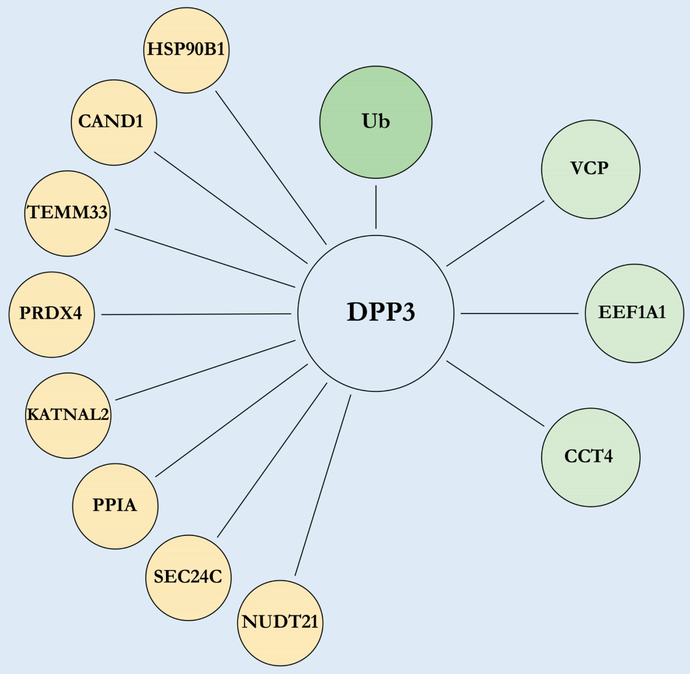

Figure 3. DPP3 interactome

SILAC-MS experiments unveiled over **30 putative interactors of DPP3**. The selection of candidate proteins was based on the SILAC-MS ratio, which compares their abundance in DPP3-expressing cells to empty vector transformed cells. Furthermore, considering the physiological roles of the identified proteins, the most promising candidates were selected for downstream experiments.

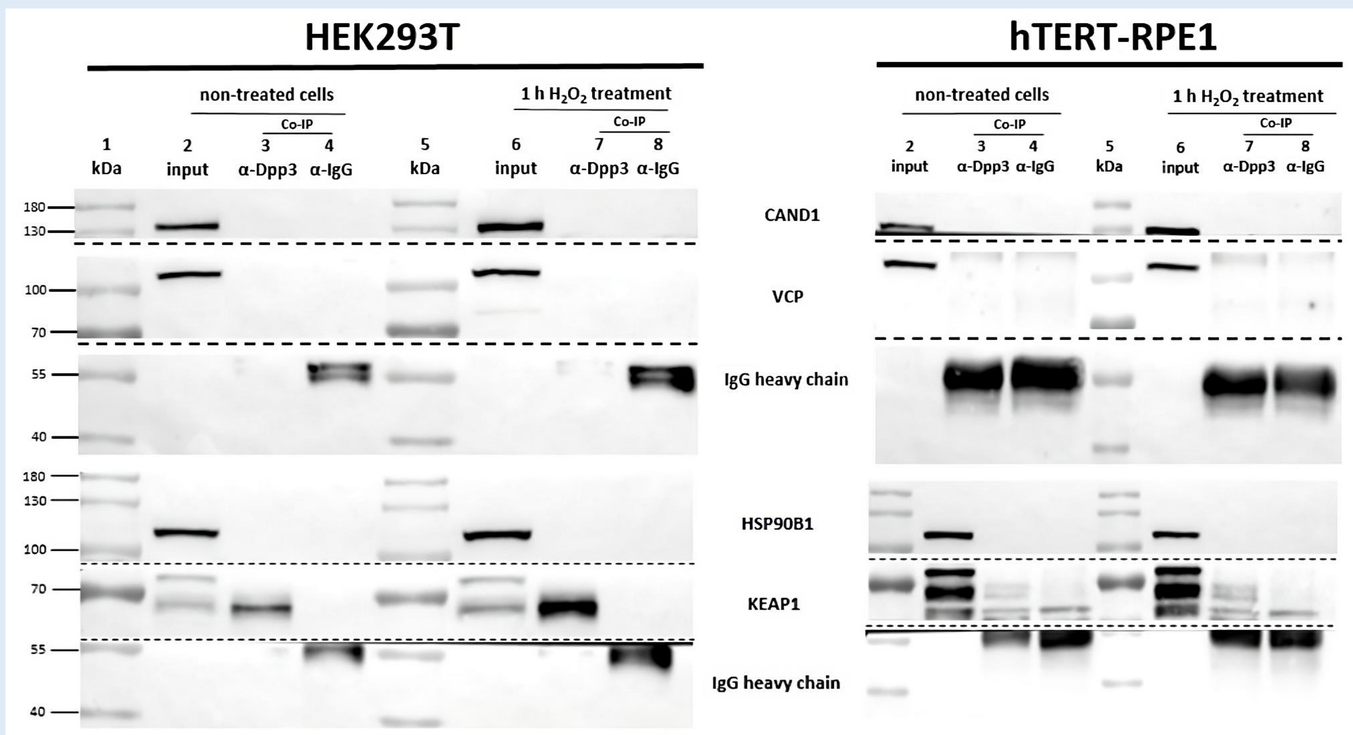

Figure 4. Putative DPP3 interactors.
Co-IP experiments were conducted using HEK293T and hTERT-RPE1 cells to confirm selected interactions, with the interaction between DPP3 and KEAP1 serving as a positive control.

CONCLUSION

The investigations of protein interactions have the potential to significantly enhance the understanding of the NRF2-KEAP1 pathway and its relevance to noncommunicable diseases (NCDs). While the putative interactors of DPP3 were not confirmed in this experiment, the exploration of novel interactors holds for uncovering new insights into the broader functions of DPP3. These findings could ultimately contribute to the identification of novel therapeutic targets for diseases associated with oxidative stress. As we currently study other potential SILAC candidates, further research is needed to fully elucidate the intricate network of protein interactions involving DPP3 and its implications for NCD pathogenesis.

ACKNOWLEDGEMENT
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