SILAC-MS analysis of human dipeptidyl peptidase 3 interactome

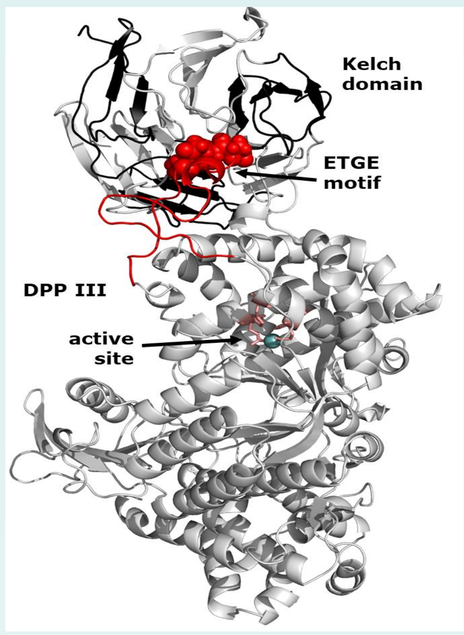
Katarzyna Kliza[1], Koraljka Husnjak[1], Mihaela Matovina[2]

[1] Goethe University School of Medicine, Institute of Biochemistry II, Frankfurt a/M, Germany

[2] Ruđer Bošković Institute, Division of Organic Chemistry and Biochemistry, Zagreb, Croatia

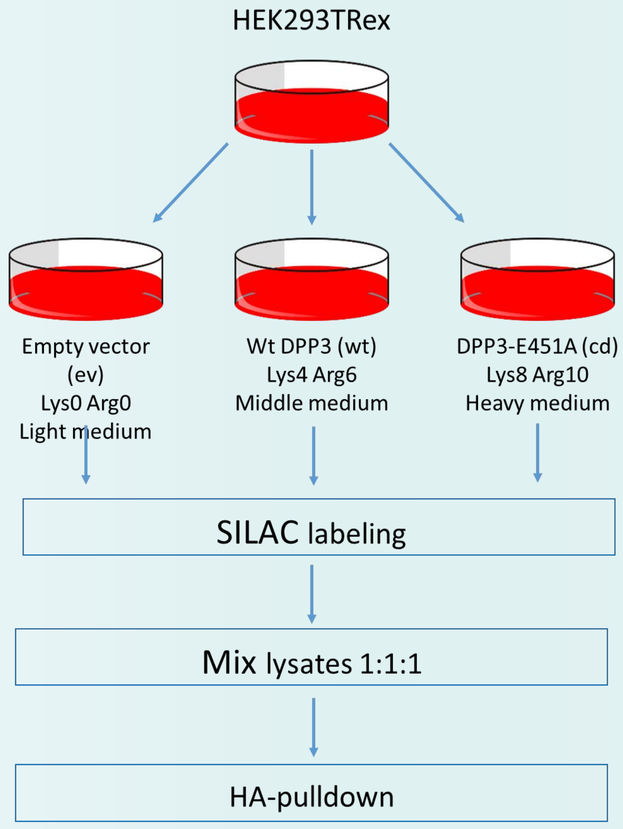
INTRODUCTION

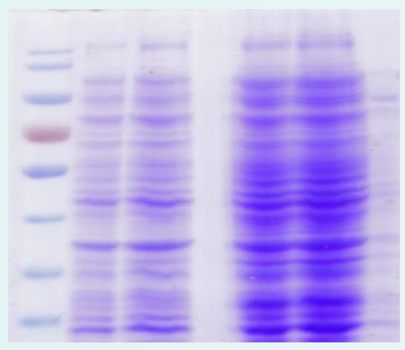
Dipeptidyl peptidase 3 (DPP3) is the zinc metallopeptidase, which cleaves dipeptides from the amino-termini of 3 to 10 amino acids long peptides. Its peptidase activity and ubiquitous presence indicate that it might have a role in the final stages of protein turnover, and human DPP3 (hDPP3) affinity and activity towards certain bioactive peptides implies its potential role in the regulation of blood pressure and pain, however, the precise physiological role of this peptidase have not been proven yet. The results of several recent studies indicate that it has a role in the regulation of oxidative stress response mediated through the interaction with Keap1 protein, the substrate adaptor for E3 ubiquitin ligase that binds the transcription factor Nrf2 in the cytoplasm, thus facilitating its ubiquitination and subsequent degradation in the basal conditions. Under oxidative stress conditions, Keap1 releases Nrf2 from the complex, enabling its translocation to the nucleus and the transcription of the whole array of genes coding for proteins involved in the oxidative stress response. One of the mechanisms that facilitates release of Nrf2 from the Keap1 complex is binding of hDPP3 to the Kelch domain of Keap1. The interaction with Keap1 is the only interaction of hDPP3 confirmed so far by several groups, including ours. In order to find other interactors of hDPP3 that could give us additional clues about its physiological role, we investigated hDPP3 interactome by SILAC-MS high-throughput approach. We found around 30 new putative inteactors of hDPP3 by this aproach, and we are currently trying to confirm selected interactors by more specific methods.

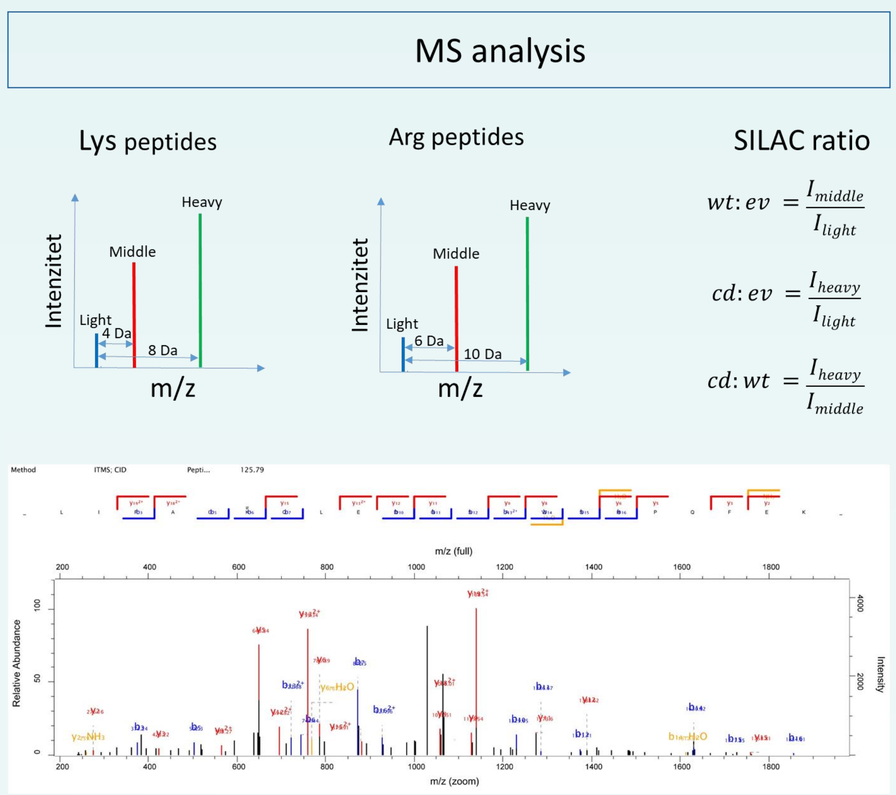


METHOD

Stable isotope labeling by amino acids in cell culture coupled to MS







RESULTS

- More than 30 putative interactors found in at least 2 out of 4 biological replicates of the experiment
- None of the putative interactors found in other studies → Keap1 (the only confirmed interactor) was found in 1 out of 4 replicates (highest WT : EV ratio 7,5)
- only one putative interactor found in all 4 replicates → RPS27A; UBB; UBC; UBA52
- 3 putative interactors found in 3 out of 4 replicates → EEF1A1, VCP, CCT4
- 10 interactors tested by yeast two-hybrid → none of the interactions was confirmed – hDPP3 part of the complex without direct interaction with only 1 protein???
- Further analysis to confirm the interaction are on the way → 6 proteins chosen
  - GST-pulldown
  - Co-IP – with overexpressed and endogenous proteins
  - Bimolecular fluorescence complementation analysis (BiFC)

STRING database analysis of putative interactors