Screening of ALDH2 interactome by combined Immunoprecipitation and Mass Spectrometry

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Introduction

- Mitochondrial aldehyde dehydrogenase 2 (ALDH2) has been shown to protect against oxidative stress in multiple organs
- ALDH2 has a role in preventing oxidative stress induced 4-HNE adduction in multiple organs
- ALDH2 may have an unidentified protein interaction network, which would help elucidate its importance in various cellular functions.
- Studies show that ALDH2 aids in protecting the lungs against oxidative stress
- We are studying ALDH2 in the lung because it is said to be critical to the health of cells and an important shield from damage occurring under oxidative stress (Chen et. al)
- Information regarding the ALDH2 interactome is limited
- To explore cells which assume different cell profiles, type II AEC and club cell, may give us new insight of ALDH2's counteractive role against oxidative stress

Objective

- To screen and identify binding partners of ALDH2

Materials and Methods

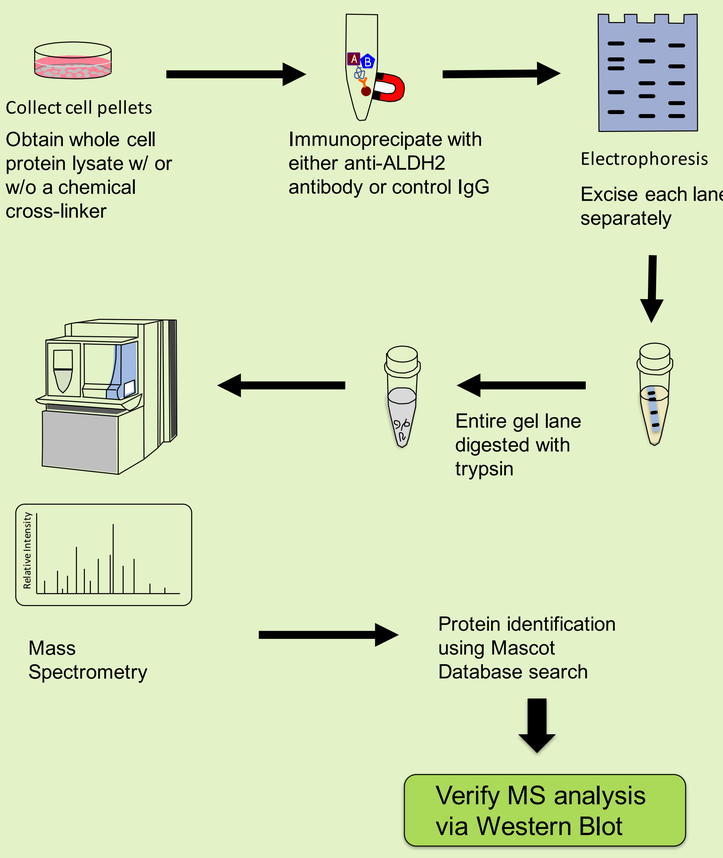
Cells : H441 cells (human lung epithelial cell line)

Cell culture medium : RPMI-1640 containing 10% FBS plus antibiotics

Immunoprecipitaion : Dynabeads™ Co-Immunoprecipitation Kit (Invitrogen)

Antibodies used : Mouse anti-ALDH2 antibody and control IgG

Data analysis : Scaffold4 (Proteome Software, Inc)



Results

Confirmation of the quality of the conducted immunoprecipitation and mass spectrometry

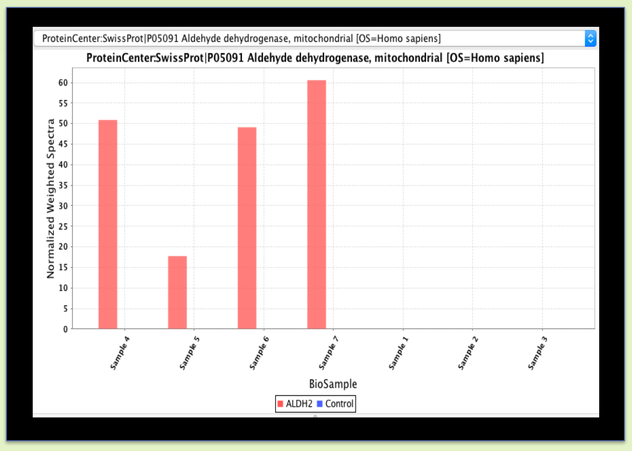


Figure 1. ALDH2 proteins are detected in only elutes form ALDH2 antibody-immunoprecipatated samples

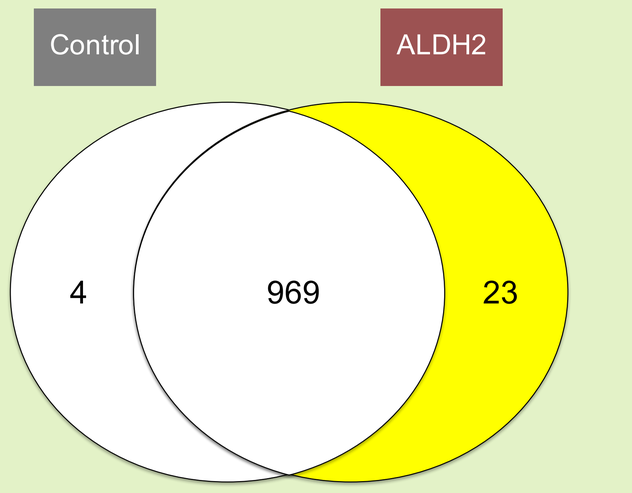


Figure 2. Overview of numbers of identified proteins in samples co-immunoprecipitated with ALDH2 antibody or control IgG. 23 proteins highlighted in yellow denote those proteins which are highly detected in ALDH2 group.

Protein Name	Gene	T-Test (p-value): *(p < 0.00141)
Isoform 2 of Protein kinase C and casein kinase substrate in neurons protein 2	PACSIN2	< 0.00010
Unconventional myosin-Ic	MYO1C	0.0013
dnaJ homolog subfamily B member 1	DNAJB1	< 0.00010
Isoform 2 of Cordon-bleu protein-like 1	COBLL1	< 0.00010
Protein-glutamine gamma-glutamyltransferase 2	TGM2	0.00089
Isoform 3 of Myosin-10	MYO10	0.00093
RNA cytidine acetyltransferase	NAT10	0.00029
Myosin phosphatase Rho-interacting protein	MPRIP	0.00031
DNA topoisomerase 2-alpha	TOP2A	0.00044
Nucleolar protein 56	NOP56	0.00091
Ankycorbin	RAI14	0.00029
Protein RRP5 homolog	PDCD11	0.00093
Guanine nucleotide-binding protein-like 3	GNL3	0.00071
Elongation factor 1-beta	EEF1B2	0.00028
Pescadillo homolog	PES1	< 0.00010
Ribosome biogenesis protein BOP1	BOP1	0.0007
Putative ATP-dependent RNA helicase DHX30	DHX30	0.0012
Probable tRNA(His) guanylyltransferase	THG1L	0.001
Nucleolar protein 11	NOL11	0.00027
RNA 3'-terminal phosphate cyclase-like protein	RCL1	0.00055
Nucleolar and coiled-body phosphoprotein 1	NOLC1	0.0011
Isoform C of AP-1 complex subunit beta-1	AP1B1	0.00011
Isoform 1 of Unconventional myosin-VI	MYO6	0.00037

p-values calculated by Student's t-test are adjusted by Benjamini Hochberg method

Figure 3. List of 23 proteins highly detected in samples co-immunoprecipitated with ALDH2 antibody.

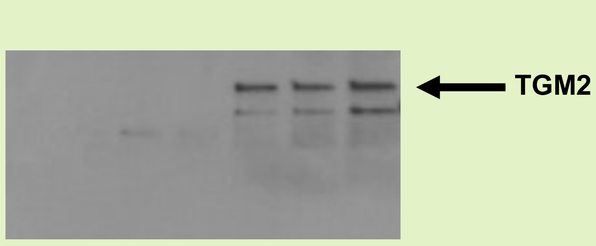


Figure 4. Control IgG- and ALDH2 antibody- IP elutes were run on Western blotting to evaluate the results of mass spectrometric analysis.

Results

- 23 distinct proteins were listed as significantly high in the ALDH2-IP group compared to controls.
- Most of 23 candidate proteins largely exist outside of mitochondria.
- The group of distinct proteins includes, but is not limited to, DNAJB1, Transglutaminase 2, and block of proliferation 1.

Conclusion

- ALDH2 may have an unidentified protein interaction network, which would help elucidate its importance in various cellular functions
- The combination of immunoprecipitation and mass spectrometry will give us insights that could not be easily attained otherwise

Future Research Plans

Further evaluation is required to elucidate the binding partners of ALDH2

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References

Chen, C.-H., et al. "Targeting Aldehyde Dehydrogenase 2: New Therapeutic Opportunities."*Physiological Reviews*, vol. 94, no. 1, Jan. 2014, pp. 1–34., doi:10.1152/physrev.00017.2013.