MDH CURE Start Up Project

Human mitochondrial and cytosolic phosphorylation

Description of the science/background for this CURE: Protein phosphorylation is a crucial post-translational modification that affects the structure and function of numerous proteins within cells. Mass spectrometry has identified several experimentally determined phosphorylation sites for both MDH isoforms. Additionally, various online phosphoprediction tools have predicted several more potential phosphorylation sites. The dysregulation of protein kinases, which control many metabolic enzymes and regulators, is implicated in various disorders, including cancer and metabolic syndrome. Surprisingly, there is a scarcity of studies investigating the impact of MDH phosphorylation, making it an intriguing area for students to explore. Phosphorylation of serine, threonine, and tyrosine residues in proteins modifies their polarity, charge, hydrogen bonding, and other non-covalent interactions, making projects focused on this subject an exciting application of structure-function relationships in a CURE. Moreover, existing mutations are available for both human mitochondrial MDH and cytosolic MDH, providing excellent resources for your CURE.

Literature sources that support this science: There are only a few published studies demonstrating the phosphorylation of mammalian MDH. Specifically, two older papers provide relatively accessible information and offer clues about potential challenges in this area.

- ***Different expressions of cooperativity in the kinetics of two forms of cytoplasmic malic dehydrogenase,*** *Vetterlein and Cassman, Biochemistry* (1974) 13:3243.

There are indications that in cancer, MDH activity and expression are increased. The following papers show some support for MDH phosphorylation and are a nice place to start discussions with students designing hypotheses:

- ***Cytosolic malic dehydrogenase activity is associated with a putative substrate for the transforming gene product of Rous sarcoma virus,*** *Rübsamen, et al., Proc. Natl. Acad. Sci USA* (1982) 79:228.
- ***Cytosolic malate dehydrogenase activity helps support glycolysis in actively proliferating cells and cancer.*** Hanse *et. al*. Oncogene Vol 36, 3915-3924 (2017).
- ***Characterization of the Role of the Malate Dehydrogenases to Lung Tumor Cell Survival***. Zhang et al. J Cancer. Vol 8., 2088-2096. 2017.

While not human, there is one study that shows the involvement of MDH phosphorylation and protein degradation in yeast. Nothing has been done similar to test what happens in human MDH.

- **Glucose-induced Degradation of the MDH2 Isozyme of Malate Dehydrogenase in Yeast.** Minard and McAlister-Henn. J. Biol Chem. Vol 267(24) pp. 17458-17464. 1992

A search of the Phosphosite.net web site was used to find phosphorylation sites of MDH. This website links to curated studies and databases of protein modifications on MDH1 and MDH2 to identify sites of post-translational modification (PTM), including phosphorylation. The number of references is plotted versus sequence, with the type of post-translational modification shown in color according to the posted legend. The site is interactive and allows the user to choose either “LTP - low throughput papers” which are published records of a phosphorylation not using LCMS; or “HTP – high throughput papers” in which LCMS was used to identify the type of post-translational modification.

3-5 Learning goals for this CURE:

- Analyze the bioinformatic and mass-spectroscopy databases to determine potential role of phosphorylation of MDH and its impact on structure and function
- Predict and design experiments using phosphomimetic mutations to investigate the changes in MDH activity, regulation or structure.
- Evaluate potential changes to MDH structure and activity using computational models for wild-type and phosphomimic mutations.

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Research question for this CURE: What is the impact of phosphorylation on the activity and structure of human MDH? There are putative phosphorylation sites at each of the key domains of MDH making this open for many different questions.

Hypothesis possibilities: Analysis of the structure with key domains of MDH (active site, interface, loop region) and the known phosphorylation sites can generate several interesting hypotheses. Some of the putative phosphorylation sites also lie in the interface of protein-protein interactions (predicted and published) for both mitochondrial and cytosolic MDH. See the MDH Members page for a few specific examples and resources to help MCC faculty guide students through hypothesis generation.

CURE format (modular, semester, or either): EITHER - If using existing mutations, this could be done as a modular cure – focused on one area of MDH function. If a full semester CURE is planned, students could conduct both enzyme assays and a possible structural analysis.

Ideal group size for this CURE: 2-4 students per group.

Ideal course/level for this CURE: Gen Chem (second semester), biochem lab 3rd/4th year, capstone course, interdisciplinary if using genetics or cell-based assays. Examples of syllabi are found on the MDHCUREs.org website.

Week by week lab activities for a modular and/or semester long version of this CURE

- 3-4 weeks on reading, visualization (PyMOL…) computational design and hypothesis development
- 3 weeks for SDM in parallel with…
- 3 weeks for expression, purification and protein assay/westernblot
- 4 weeks for testing hypothesis – enzyme assay, protein stability, SEC, detailed kinetic analysis

PyMOL or other rendering site worksheet/assignment, Site Directed Mutagenesis (if not using existing mutants), protein expression and purification, enzyme assay, possible structural assay

Instrumentation/equipment/key reagents needed for this CURE: Thermocycler (if doing SDM), incubator for expression (room temp or 37°C), centrifuge (min 10K x g; optimal speed 30Kxg), 2 ml IMAC beads and column/batch chromatography– 500 ml culture will produce >2-5 mg of purified protein, spectrophotometer capable of 340 nm UV measurements. One per group for real time assays or stop time assay using plate reader.

Bacterial Expression: Both wild-type and mutant human MDH clones express well after a 3-4 hour 37°C or an overnight 20°C IPTG induction in BL21(DE3) cells. A 500 ml culture should yield between 10 and 20 mg of purified protein. Both mitochondrial and cytosolic MDH are protein is stable but should be diluted to 1.2 mg/ml (determined by Bradford) for short term storage. Failure to do so will result in aggregation/precipitation. Long term storage should follow instructions on MDH Storage

Protein (WT and/or specific mutant), organism: Details on these and other clones can be found in the MDH Members page
Cytosolic (hMDH1) is expressed as several N-terminal splice variant forms.

- hMDH1V3 (human cytosolic MDH splice variant 3) and mutants S108D, S236D, 328D
- hMDH2 (human mitochondrial MDH) and mutants S8D, S46D, Y56D, S85D, S222D, S224D
    - mito MDH numbering does NOT include the mitochondrial targeting peptide.

Plasmids needed and where to obtain them: J Provost

MW/pI/ extinction coefficient (280nm) of protein (WT and/or specific mutant): *Additional detailed information can be found in MDH Members page*

hMDH1V3	hMDH2
- 352 aa (386 with His tag), - Monomer MW: 40,601 Da, - Extinction Coefficient (280 nm predicted based from sequence) 35,410 M^{-1} cm^{-1} - 280 nm Absorbance, 0.1% 0.87 - pI 6.83 - Charge at pH 7.0 (-1.36)	- 314 aa (324 with His tag), - Monomer MW: 34,806 Da, - Extinction Coefficient (280 nm predicted based from sequence) 8,940 M^{-1} cm^{-1} - 280 nm Absorbance, 0.1% 0.26 - pI. 7.11 - Charge at pH 7.0 (1.08)

PDB ID: ***hMDH1:*** 7RM9 (human MDH), 5MDH (porcine cytosolic MDH with NAD and OAA analogue), MDHC_HUMAN (AlphaFold predicted model using 5MDH with NAD and MAK (OAA analogue)).

NOTE: the extra amino acids of hMDH1V3 are not yet solved, these structures are those without the extended N tail on this splice varient.

hMDH2: 4WLN, apo MDH, 4WLE (with citrate), 4WLF (with malate), 4WLO (with NADH and OAA), 2DFD (with malate and NAD).