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Proteomics sample preparation

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We use this protocol and it's working

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Abstract

Protocol for proteomic analysis and data acquisition used in: "Selective Deficiency of Mitochondrial Respiratory Complex I Subunits Ndufs4/6 Causes Tumor Immunogenicity". The paper's abstract is as follows:

Cancer cells frequently rewire their metabolism to support proliferation and evade immune surveillance, but little is known about metabolic targets that could increase immune surveillance. Here we show a specific means of mitochondrial respiratory Complex I (CI) inhibition that improves tumor immunogenicity and sensitivity to immune checkpoint blockade (ICB). Targeted genetic deletion of either *Ndufs4* or *Ndufs6*, but not other CI subunits, induces an immune-dependent growth attenuation in melanoma and breast cancer models. We show that deletion of *Ndufs4* induces expression of the MHC class-I co-activator *Nlrc5* and antigen presentation machinery components, most notably *H2-K1*. This induction of MHC-related genes is driven by a pyruvate dehydrogenase-dependent accumulation of mitochondrial acetyl-CoA, which leads to an increase in histone H3K27-acetylation within the *Nlrc5* and *H2-K1* promoters. Taken together, this work shows that selective CI inhibition restricts tumor growth, and that specific targeting of *Ndufs4* or *Ndufs6*, increases T-cell surveillance and ICB responsiveness.

Troubleshooting

Protein digestion and isobaric labelling for mass spectrometry analysis

1 Was performed as previously described

Citation

Perry EA, Bennett CF, Luo C, Balsa E, Jedrychowski M, O'Malley KE, Latorre-Muro P, Ladley RP, Reda K, Wright PM, Gygi SP, Myers AG, Puigserver P (2020). Tetracyclines promote survival and fitness in mitochondrial disease models..

https://doi.org/10.1038/s42255-020-00334-y LINK

Briefly tumor samples from allograft study were lysed with 4 mL of SDS lysis buffer (2.0% SDS (w/v), 250 mM NaCl, 5 mM TCEP, EDTA-free protease inhibitor cocktail (Promega), and 100 mM HEPES, pH 8.5) using an Omni tissue homogenizer. Extracts were reduced at 57°C for 30 min and cysteine residues were alkylated with iodoacetamide (14 mM) in the dark at room temperature for 45 min. Extracts were purified by methanol–chloroform precipitation and pellets were washed with ice-cold methanol. Pellets were
resuspended in 2 mL of 8 M urea (containing 50 mM HEPES, pH 8.5) and protein concentrations were measured by BCA assay (Thermo Fisher Scientific) before protease digestion. 100 µg of protein were diluted to 4 M urea and digested overnight with 4 µg LysC (Wako). Digests were diluted further to a 1.5 M urea concentration and 5 µg of trypsin (Promega) was added for 6 hours at 37°C. Digests were acidified
with 50 µl of 20% formic acid and subsequently desalted by C18 solid-phase extraction (50 mg, Sep-Pak, Waters). Digested peptides were resuspended in 100 µl of 200 mM HEPES, pH 8.0. 10 µL of TMTpro reagents (Thermo Fisher Scientific) was added to each solution for 1 hour at room temperature (25°C). After incubating, the reaction was quenched by adding 4 µL of 5% (w/v) hydroxylamine. Labelled peptides were combined and subsequently desalted by C18 solid-phase extraction (50 mg, Sep-Pak, Waters) before basic pH reversed- phase separation.

Basic pH reverse-phase separation for mass spectrometry analysis

2 Was performed as previously described

Citation

Perry EA, Bennett CF, Luo C, Balsa E, Jedrychowski M, O'Malley KE, Latorre-Muro P, Ladley RP, Reda K, Wright PM, Gygi SP, Myers AG, Puigserver P
(2020). Tetracyclines promote survival and fitness in mitochondrial disease models..

https://doi.org/10.1038/s42255-020-00334-y LINK

Briefly, tandem mass tag (TMT)-labeled peptides were solubilized in 500 µL solution containing 5% acetonitrile in 10 mM ammonium bicarbonate, pH 8.0 and separated by an Agilent 300 Extend C18 column (5 mm particles, 4.6 mm inner diameter and 220 mm in length). An Agilent 1100 binary pump coupled with a photodiode array detector (Thermo Fisher Scientific) was used to separate the peptides. A 40-min linear gradient from 20% to 40% acetonitrile in 10 mM ammonium bicarbonate, pH 8 (flow rate of 0.6 ml/min) separated the peptide mixtures into a total of 96 fractions (30 sec). A total of 96 fractions was consolidated into 12 samples in a checkerboard fashion, acidified with 20 µl of 20% formic acid and vacuum dried to completion. Each sample was re-dissolved in 5% formic acid, 5% ACN, desalted via StageTips before liquid chromatograph–tandem mass spectrometry (LC–MS/MS) analysis. LC–MS/MS analysis. Data were collected using an Orbitrap Fusion Lumos mass spectrometer (Thermo Fisher Scientific, San Jose, CA) coupled with a Proxeon EASY-nLC 1200 LC pump (Thermo Fisher Scientific). Peptides were separated on a 100 µm inner diameter microcapillary column packed with 35 cm of Accucore C18 resin (2.6 µm, 100 Å, Thermo Fisher Scientific). Peptides were separated using a 3 hours gradient of 6–22% acetonitrile in 0.125% formic acid with a flow rate of ~400 nL/min. Each analysis used an MS3-based TMT method as described previously described

Citation

McAlister GC, Nusinow DP, Jedrychowski MP, Wühr M, Huttlin EL, Erickson BK, Rad R, Haas W, Gygi SP
(2014)
. MultiNotch MS3 enables accurate, sensitive, and multiplexed detection of differential expression across cancer cell line proteomes..

https://doi.org/10.1021/ac502040v LINK

. The data were acquired using a mass range of m/z 400 – 1400, resolution at 120,000, AGC target of 1×10^6, a maximum injection time 100 ms, dynamic exclusion of 180

seconds for the peptide measurements in the Orbitrap. Data dependent MS 2 spectra were acquired in the ion trap with a normalized collision energy (NCE) set at 35%, AGC target
set to 2.0 × 10^5 and a maximum injection time of 120 ms. MS3 scans were acquired in the Orbitrap with an HCD collision energy set to 45%, AGC target set to 1.5 × 10^5, maximum injection time of 200 ms, resolution at 50,000 and with a maximum synchronous precursor selection (SPS) precursors set to 10.

Mass spectrometry data processing and spectra assignment

3 Was performed as previously described

Citation

Perry EA, Bennett CF, Luo C, Balsa E, Jedrychowski M, O'Malley KE, Latorre-Muro P, Ladley RP, Reda K, Wright PM, Gygi SP, Myers AG, Puigserver P
(2020). Tetracyclines promote survival and fitness in mitochondrial disease models..

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Briefly, in-house developed software was used to convert acquired mass spectrometric data from the .RAW file to the mzXML format. Erroneous assignments of peptide ion charge state and monoisotopic m/z were also corrected by the internal software. SEQUEST algorithm was used to assign MS2 spectra by searching the data against a protein sequence database including Mouse Uniprot Database (downloaded June 2018) and known contaminants such as mouse albumin and human keratins. A forward (target) database component was followed by a decoy component including all listed protein sequences. Searches
were performed using a 20ppm precursor ion tolerance and requiring both peptide termini to be consistent with trypsin specificity. 16-plex TMT labels on lysine residues and peptide N termini (+304.2071 Da) were set as static modifications and oxidation of methionine residues (+15.99492 Da) as a variable modification. An MS2 spectra assignment false discovery rate (FDR) of less than 1% was implemented by applying the target-decoy database search strategy. Filtering was performed using a linear discrimination analysis method to create one combined filter parameter from the following peptide ion and MS2 spectra properties: XCorr and ΔCn, peptide ion mass accuracy, and peptide length. Linear discrimination scores were used to assign probabilities to each MS2 spectrum for being assigned correctly and these probabilities were further used to filter the data set with an MS2 spectra assignment FDR to obtain a protein identification FDR of less than 1%.

Determination of TMT reporter ion intensities

4 Was performed as previously described

Citation

Perry EA, Bennett CF, Luo C, Balsa E, Jedrychowski M, O'Malley KE, Latorre-Muro P, Ladley RP, Reda K, Wright PM, Gygi SP, Myers AG, Puigserver P
(2020). Tetracyclines promote survival and fitness in mitochondrial disease models..

https://doi.org/10.1038/s42255-020-00334-y LINK

Briefly, for reporter ion quantification, a 0.003 m/z window centered on the theoretical m/z value of each reporter ion was monitored for ions, and the maximum intensity of the signal to the theoretical m/z value
was recorded. Reporter ion intensities were normalized by multiplication with the ion accumulation time for each MS2 or MS3 spectrum and adjusted based on the overlap of isotopic envelopes of all reporter ions. Following extraction of the reporter ion signal, the isotopic impurities of the TMT reagent were corrected using the values specified by the manufacturer's specification. Total signal-to-noise values for all peptides were summed for each TMT channel and all values were adjusted to account for variance and a total minimum signal-to-noise value of 200 was implemented.

Citations

Step 1

Perry EA, Bennett CF, Luo C, Balsa E, Jedrychowski M, O'Malley KE, Latorre-Muro P, Ladley RP, Reda K, Wright PM, Gygi SP, Myers AG, Puigserver P. Tetracyclines promote survival and fitness in mitochondrial disease models.
https://doi.org/10.1038/s42255-020-00334-y

Step 2

Perry EA, Bennett CF, Luo C, Balsa E, Jedrychowski M, O'Malley KE, Latorre-Muro P, Ladley RP, Reda K, Wright PM, Gygi SP, Myers AG, Puigserver P. Tetracyclines promote survival and fitness in mitochondrial disease models.
https://doi.org/10.1038/s42255-020-00334-y

Step 2

McAlister GC, Nusinow DP, Jedrychowski MP, Wühr M, Huttlin EL, Erickson BK, Rad R, Haas W, Gygi SP. MultiNotch MS3 enables accurate, sensitive, and multiplexed detection of differential expression across cancer cell line proteomes.
https://doi.org/10.1021/ac502040v

Step 3

Perry EA, Bennett CF, Luo C, Balsa E, Jedrychowski M, O'Malley KE, Latorre-Muro P, Ladley RP, Reda K, Wright PM, Gygi SP, Myers AG, Puigserver P. Tetracyclines promote survival and fitness in mitochondrial disease models.
https://doi.org/10.1038/s42255-020-00334-y

Step 4

Perry EA, Bennett CF, Luo C, Balsa E, Jedrychowski M, O'Malley KE, Latorre-Muro P, Ladley RP, Reda K, Wright PM, Gygi SP, Myers AG, Puigserver P. Tetracyclines promote survival and fitness in mitochondrial disease models.
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