MICAL1 monoxygenase in breast cancer: target validation and assay optimization.

A Data Management Plan created using DMP Assistant

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Project abstract:

The MICAL1 monooxygenase is has recently emerged as an important regulator of the actin cytoskeleton, with prominent roles in cytokinesis and endocytosis. Analysis of genomic databases has revealed significant associations of elevanted MICAL1 expression with a variety of cancer types including advanced forms of breast cancer, and poor patient outcomes in breast and renal cancers. To determine if breast cancers were dependent on MICAL1, CRISPR/Cas9 was used to knockout MICAL1 expression in MDA MB 231 cells. When grown as orthotopic xenograft tumours in immunocompromised mice, knockout cells yielded slow growing tumours with evidence of increased apoptosis and macrophage infiltration. These results support MICAL1 as a potential therapeutic target. The catalytic monoxygenase and calponin homology domains of MICAL1 were expressed and purified from E. coli cells. Recombinant protein was used to establish an vitro biochemical assay of MICAL1 activity based on hydrogen peroxide generation detected by a colour change of Amplex Red, which yielded a Z' prime value of 0.70. A tool compound demonstreated dose-dependent MICAL1 inhibition in the Amplex Red assay, supporting its utility as a biochemical screen. A secondary screen of MICAL1 activity was established, using the fluorescence of pyrene-labelled actin as a read-out of actin polymerization. The tool compound also demonstrated dose-dependent inhibition in this assay, supporting its utility as an independent counter screen. An orthogonal screen that would reveal if compounds acted as hydrogen peroxide scavengers was also set-up to identify possible false positives from the initial screen. In this application, five target validation aims are proposed. Using validated single guide RNAs, CRISPR/Cas9 will knockout MICAL1 in a panel of breast cancer cell lines. The ability of knockout versus control cells to grow as 2D monolayers and 3D spheroids will be assessed by live cell imaging. Futhermore, orthotopic xenograft tumour assays will provide robust in vivo validation results. The effects of MICAL2 and MICAL3 knockout will be compared to the consequences of MICAL1 deletion to determine whether these related proteins have similar functions, which will help define the importance of compound selectivity. Mechanism of action studies will reveal if MICAL1 inhibition would sensitize cells to pro-apoptotic stimuli through NDR1 activation. Additional bioinformatic studies will reveal additional associations between MICAL1 expression and cancer, with a particular focus on tumour suppressors and oncogenes to identify biomarkers for future patient selection. In the second part of the application, the established biochemical assays of hydrogen peroxide production and NADPH consumption will be optimized. Recombinant protein expression and purification will be improved. Assays conditions will be varied to improve signal to noise and Z' values. The current 96 well assay format will be transferred to 384 wells, and optimization will be carried out at the SMART screening facility to enable transfer to HTS instrumentation. Secondary and orthogonal assays will enable hit selection and prioritization. At the end of this proposal, a validated package of robust target validation and primary/secondary/orthogonal/cell-based screens will enable the start of library screening for a first-in-class small molecule MICAL1 inhibitor.

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Responsibilities and Resources

Who will be responsible for data management? Will the Principal Investigator (PI) hold all responsibility during and beyond the project, or will this be divided among a team or partner organizations?

Data management will be the responsibility of the principal investigator Michael Olson

In the event that the PI leaves the project, who will replace them? Who will take temporary responsibility until a new PI takes over?

Not applicable.

List all expected resources for data management required to complete your project. What hardware, software and human resources will you need? What is your estimated budget?

Cloud storage for archived data (e.g. OneDrive) $12.80 per user per month. For 4 users over 5 years = $3,072.

Data Collection

What types of data will you collect, create, link to, acquire and/or record?

Microscopy images, western blots, multi-well plate readers, spreadsheets

Answer the following regarding file formats:

A. **What file formats do you expect to collect (e.g. .doc, .csv, .jpg, .mov)?**
B. **Are these file formats easy to share with other researchers from different disciplines?**
C. **In the event that one of your chosen file formats becomes obsolete (or is no longer supported) how will you ensure access to the research data?**
D. **Does your data need to be copied to a new media or cloud platform, or converted to a different file format when you store or publish your datasets?**

Question not answered.

Answer the following regarding naming conventions:

A. **How will you structure, name and version-control your files to help someone outside your research team understand how your data are organized?**
B. **Describe your ideal workflow for file sharing between research team members step-by-step.**
C. **What tools or strategies will you use to document your workflow as it evolves during the course of the project?**

Question not answered.

Documentation and Metadata

What support material and documentation (e.g. ReadMe) will your team members and future researchers need in order to navigate and reuse your data without ambiguity?

Question not answered.

How will you undertake documentation of data collection, processing and analysis, within your workflow to create consistent support material? Who will be responsible for this task?

Question not answered.

Do you plan to use a metadata standard? What specific schema might you use?

Question not answered.

How will you make sure that a) your primary data collection methods are documented with transparency and b) your secondary data sources (i.e., data you did not collect yourself) — are easily identified and cited?

Question not answered.

Storage and Backup

List your anticipated storage needs (e.g., hard drives, cloud storage, shared drives). List how long you intend to use each type and what capacities you may require.

Question not answered.

What is your anticipated backup and storage schedule? How often will you save your data, in what formats, and where?

Question not answered.

Keeping ethics protocol review requirements in mind, what is your intended storage timeframe for each type of data (raw, processed, clean, final) within your team? Will you also store software code or metadata?

Question not answered.

Sharing, Reuse, and Preservation

How will your data (both raw and cleaned) be made accessible beyond the scope of the project and by researchers outside your team?

Question not answered.

Is digital preservation a component of your project and do you need to plan for long-term archiving and preservation?

Question not answered.

What data will you be sharing publicly and in what form (e.g. raw, processed, analyzed, final)?

Question not answered.

Have you considered what type of end-user license to include with your data?

Question not answered.

What tools and strategies will you take to promote your research? How will you let the research community and the public know that your data exists and is ready to be reused?

Question not answered.

Ethics and Legal Compliance

Are there institutional, governmental or legal policies that you need to comply with in regards to your data standards?

Question not answered.

Will you encounter protected or personally-identifiable information in your research? If so, how will you make sure it stays secure and is accessed by approved team members only?

Question not answered.

Before publishing or otherwise sharing a dataset are you required to obscure identifiable data (name, gender, date of birth, etc), in accordance with your jurisdiction's laws, or your ethics protocol? Are there any time restrictions for when data can be publicly accessible?

Question not answered.