An Omics Approach to Identify Model-agnostic Disease-driving Nodes in AKI: Implications for Drug Development

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Translational Medicine

RESEARCH PLAN

A.RATIONALE

Acute Kidney Injury (AKI) is defined as the abrupt loss in kidney function caused by either injury or impairment. AKI can be caused by decreased blood flow, which can be caused by overuse of pain medication, heart failure, severe allergy or major surgery. AKI can be induced by mercury chloride, folic acid and domoic acid. Mercury is a toxic heavy metal which comes in both organic and inorganic forms. Mercury toxicity significantly affects the kidneys and can be caused by fish consumption or dental amalgam (Dhanapriya et al, 2016). Many times within Asian communities, mercury chloride is seen as an at home remedy. Folic Acid is a synthetic dietary supplement. It is often present in artificially enriched foods and pharmaceutical vitamins. Many times, vitamins containing folic acid are recommended to pregnant women (Greenberg et al, 2011). Domoic acid is naturally produced in phytoplankton and accumulates in seafood during harmful algae blooms (Ferriss et al, 2017).

There have been lots of disparities surrounding AKI and its diagnosis. In order to identify the severity of cases of AKI, Acute Tubular Necrosis (ATN) scoring is utilized. AKI could either be categorized into pre-renal AKI or ATN. Clinical trials in AKI are often punctuated with failures. Lack of robust translational success can at least in part be explained by the fact model systems might not fully recapitulate human AKI. ATN, the hallmark pathological feature of AKI, might not necessarily be governed by the same pathway in animal models in comparison to human subjects. This study examines if ROCK2 expression correlates with ATN scoring in induced AKI in mice.

B. RESEARCH QUESTION

How does ROCK2 expression correlates with ATN scoring?

- *Hypothesis*

  As the severity of AKI increases (higher ATN score), the level of ROCK2 expression will increase.

- *Expected Outcome*

  There will be higher level of ROCK2 transcripts in tissues with high ATN scores

C. PROCEDURE

Prepared hematoxylin-eosin-stained kidney slides will be obtained from mice treated $HgCl_2$, folic acid or domoic acid (IACUC #2016-004). These slides were already prepared by mentor. These slides will be examined using a compound light microscope and will be into sorted five categories based on the size of their urinary casts, seen as hyaline and granular casts in AKI. The size of the cast will be measured by Image J. The largest-sized casts will receive a score of 5 and the smallest received a score of 0 or 1. These scores should mirror Acute Tubular Necrosis (ATN) Scores (Perezella et al, 2008). A *t*-test was used to determine significance between measured values of the Sham (Control) and AKI groups.

Upon determining the presence of AKI, RT-PCR will be performed on RNA isolated from frozen tissue (IACUC #). RNA was already isolated by mentor and stored in freezer. RNA was used for other purposes than this study. RNA will be reversed transcribed and the ROCK2 gene will be amplified using qPCR.

The procedure followed will be according to manufacturer's instruction in the high capacity cDNA Reverse Transcription Kit (Qiagen, Cat #: 204054). 500 ng of RNA will be

reverse transcribed and amplified using the Power-Up SYBR Green Master Mix. The primers for PCR: Forward: TGGCCCAGTTTGCATCTTTC and Reverse: AGCAAGTTGTGTTCCCAACC

RISK AND SAFETY

All chemicals will be handled carefully using tools which will be thoroughly cleaned. Hands will be washed before and after experimentation. Proper PPE (gloves, safety goggles, lab coats, fume hood) will be used. All waste will be collected by Angion Biomedica and be properly disposed of.

Chemicals in the cDNA Reverse Transcription Kit (Qiagen, Cat #: 204054)

- Buffer RLT: Harmful is swallowed, causes severe skin burns and eye damage and harmful to aquatic life with long lasting effects. Gloves, goggles and body protection.
- Buffer RPE: May cause skin irritation. Gloves, goggles and body protection .
- Buffer RW1: Causes serious eye damage, may cause skin irritation in susceptible persons. Gloves, goggles and body protection.
- Multiscribe Reverse Transcriptase: Not Hazardous. Gloves, goggles and body protection.
- 10x RT Buffer: Not Hazardous. Gloves, goggles and body protection.
- dNTP Mix: Not Hazardous. Gloves, goggles and body protection.
- 10x RT Random Primers: Not Hazardous. Gloves, goggles and body protection.
- PowerUp SYBR Green Master Mix: Not Hazardous. Gloves, goggles and body protection.

List of Sources of Safety Information:

- SDS from ThermoFisher Scientific

- SDS from Qiagen

DATA ANALYSIS

Image J will be used to measure the size of the casts from the prepared slides. Prism Graphpad software will be used to determine significance between the size of the casts in various treatment groups. A t-test will also be used to determine significance between measured values of the Sham and AKI groups. A Pearson Product Moment Correlation was used to determine the strength of the association between the ROCK2 gene queried and the increase in Bowman's Space. A p-value ≤ 0.05 will be considered to be statistically significant

D. BIBLIOGRAPHY

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ADDENDUM

No changes were made to the Research Plan