Grant Writing & Funding Opportunities in Research

Amirali Banani
University of Washington

STEM Quest Final Lesson
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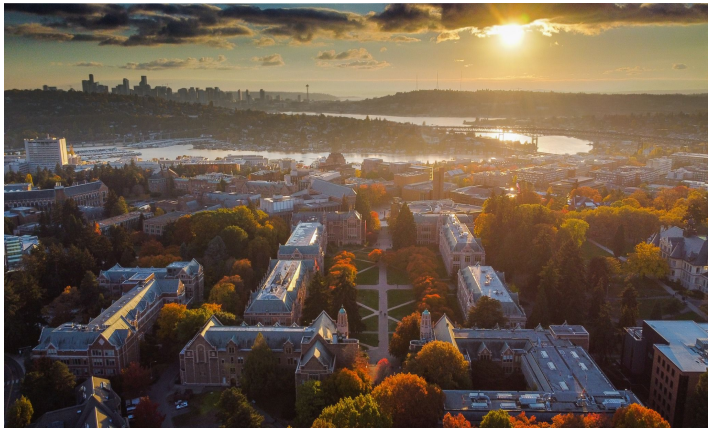
Who am I?

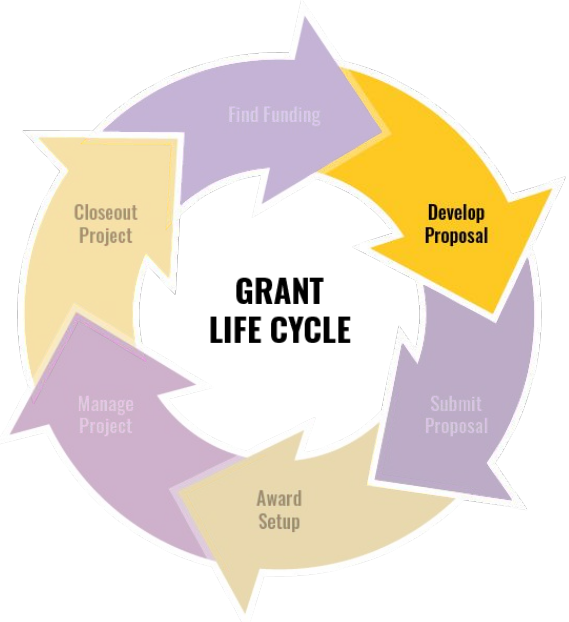
- Grew up in Vancouver, Canada 🇨🇦
- Freshman at University of Washington - Seattle studying Neuroscience
- Founded Youth STEM Initiative in 2023
- Lead Editor for Insights of Nature publication on Medium
- Interested in medicine (particularly ophthalmology), strong passion for research



YOUTH STEM INITIATIVE

Why Do Researchers Write Grant Proposals?

- Obtain funding and secure resources for research
- Establish credibility & collaboration
- Train the next generation of scientific researchers
- Promotes accountability
- Storytelling > technical docs



How to Write Grant Proposals...The NIH Way

Specific Aims → what you aim to do with your research

Background → provide some info on the topic underlying your study

Approach → how you aim to investigate through the specific aims (the procedure)

Citations → ALWAYS CITE YOUR SOURCES!!

HARVARD Summer School

National Institutes of Health – Mock Grant Proposal

The Use of Hypomethylating Agents as a Therapeutic Against DNA Hypermethylation in ccRCC || By Amirali Banani

Harvard Pre-College 2022 — Epigenetics & Gene Regulation

Dr. Tsurumi

Specific Aims

According to the results of numerous research studies, DNA hypermethylation is linked to the development of clear cell renal cell carcinomas (ccRCC) [1]. It downregulates cell cycle control and DNA damage repair genes and upregulates tumor cell invasion and metastasis by activating oncogenes in renal cells [2]. The main factor that causes ccRCC, however, is the hypermethylation of the promoter and enhancer regions in CpG islands, which can result in the inactivation of important tumor suppressor genes in clear cell renal cells known as Von Hippel-Lindau (VHL) [3]. Therefore, DNA hypermethylation is essentially involved in the transcriptional silencing of these genes. The silencing of the VHL tumor suppressor genes is caused by an excessive number of methyl groups blocking the genes from transcription factors, which results in an absence of tumor suppressor proteins and therefore contributes to carcinogenesis.

I hypothesize that, with the use of hypomethylating agents, ccRCC could be cured or prevented as these agents have been shown to inhibit aberrant cell proliferation and migration of renal cells both in in vitro and xenograft studies. Hypomethylating agents especially play a significant role in reactivating the transcriptional activity of VHL tumor suppressor genes, which is a key reversal mechanism that can overturn the effects of hypermethylation in clear cell renal cells. As a key part of my research, I will also be investigating whether hypomethylating agents are effective in demethylating important H3K4me1 marks in kidney-specific enhancer regions to reactivate the transcriptional activity of VHL tumor suppressor genes. Therefore, this proposal aims to investigate whether hypomethylating agents could potentially be used as a valid therapeutic agent against ccRCC.

Specific Aims

- Investigate the impact of [research] on [specific outcome or mechanism]
- Explain the molecular mechanisms that underlie [your research focus]
- Develop and test [novel intervention or approach] for [research focus]
- Assess the long-term effects of [intervention] on the [outcome of research focus]

Specific Aim 1: Investigate the precise epigenetic mechanisms by which hypomethylating agents counteract the effects of hypermethylation in ccRCC.

1a: Determine to what extent and at which precise locations along the gene body hypomethylating agents reactivate VHL tumor suppressor genes by the demethylation of H3K4me1 marks.

1b: Use of hypomethylating agents in the downregulation of overactive Wnt Pathways by reactivation of the Wnt antagonist, WIF-1

1c: Use of RT-qPCR to determine the efficacy of hypomethylating agents in reducing cell cycle-related gene expression by downregulation of the Wnt Pathways.

1d: Assess the difference in the quantity of proteins produced between tumor suppressor and oncogenes with the use of Western Blotting.

Specific Aim 2: Investigate the effects of hypomethylating agents on kidney physiology.

2a: Assess the change in renal cell and overall kidney function after the administration of hypomethylating agents.

2b: Compare and contrast the differences in symptoms and prognostic outcomes between patients receiving a placebo and patients receiving hypomethylating agents as therapy for ccRCC.

Background

- The significance of the problem
- Current state of knowledge
- Gaps and opportunities
- Rationale for the study
- **Remember: TELL A STORY!!**

Background

A study conducted on the role that alterations in DNA methylation play in ccRCC found that the hypermethylation of promoter regions in numerous tumor suppressor genes either partially or entirely silenced the genes [2]. This hypermethylation results in the addition of many methyl groups to CpG dinucleotides which represses the transcriptional activity of VHL tumor suppressor genes by preventing the binding of transcription factors [2]. Hypermethylation of these tumor suppressor genes in ccRCC is caused by mutations in DNA methyltransferase-1 (DNMT1) enzymes, which have been shown to increase cell proliferation, reduce apoptosis, increase colony formation and invading ability, and enhance cell migration [4]. The study used a HELP (HpaII tiny fragment Enrichment by Ligation-mediated PCR) assay to analyze genome-wide patterns of DNA methylation in ccRCC at a high resolution of 1.3 million CpG nucleotides [2]. The researchers of the study found that most of the hypermethylation occurred in renal-specific enhancer regions of the gene body that were linked to H3K4me1 marks [2]. The chromatin modification of these H3K4me1 marks at the active enhancer regions occurs when they are demethylated [2]. Chromatin modification leads to the transcriptional activation of various genes – including tumor suppressor genes [5]. This means that hypomethylating agents are able to restore the function of VHL tumor suppressor genes by removing methyl groups that are bonded to H3K4me1 marks, which allows for chromatin modification and therefore transcriptional activation of the genes. Furthermore, MOTIF analysis of abnormally hypermethylated enhancer regions revealed enrichment of DNMT1 for binding sites of the transcription factors that are normally activated under hypoxic conditions, namely: AP2a, AHR, HAIRY, ARNT, and HIF-1 [2]. This indicates that dysregulated hypoxia may play a rather important role in signaling pathways that lead to the aforementioned epigenetic changes in ccRCC. The functional importance of this aberrant hypermethylation was demonstrated by selective sensitivity of ccRCC cells to low levels of DNA methyltransferase inhibitors. Many accompanying studies found that key players of the Wnt and TGF-beta pathways, negative cell-cycle regulators, and pro-apoptotic genes have also been shown to be epigenetically silenced by hypermethylation mechanisms in ccRCC [6] – which contributes further to the development and progression of the malignancy.

Approach

- Experimental design and methods
- Data collection & analysis
- Anticipated challenges and how you plan to overcome them
- Innovation & significance of methods
- DETAIL & SPECIFICITY IS IMPORTANT!!

Approach

Specific Aim 1: Investigate the precise epigenetic mechanisms by which hypomethylating agents counteract the effects of hypermethylation in ccRCC.

The objective of this aim is to determine the precise epigenetic mechanisms by which hypomethylating agents counteract the effects of hypermethylation in clear cell renal cell carcinomas. Part of this aim is research-based, while the other part is application-based. The research aspect encompasses the discovery of how hypomethylating agents recognize and remove methyl groups from VHL tumor suppressor genes in the gene body of the renal cells. When an understanding of the molecular mechanism is acquired, this knowledge can be applied to design hypomethylating agents with specialized methyl binding sites and conformations that allow them to remove methyl groups from VHL tumor suppressor genes and prohibit them from acting upon oncogenes to prevent their unwanted transcriptional activation.

Determine to what extent and at which precise locations along the gene body hypomethylating agents reactivate VHL tumor suppressor genes by the demethylation of H3K4me1 marks.

The mechanism by which hypomethylating agents reactivate tumor suppressor genes silenced by the hypermethylation of enhancer/promoter CpG regions will be determined using Methylated DNA Immunoprecipitation (MeDIP). In this process, DNA will be extracted from the VHL tumor suppressor genes of the clear cell renal cells and purified. The purified DNA will then be sonicated into multiple smaller fragments (each between 400 and 600 base pairs in length) to improve the resolution during imaging, enhance the efficiency of immunoprecipitation, among other things. To enhance the binding affinity of the antibodies to the methyl groups bound to the DNA, the DNA fragments will be further denatured into single-stranded DNA. After denaturation is performed, the methylated DNA will be incubated with monoclonal 5-methylcytosine (5mC) antibodies at 4° celsius overnight. This allows the monoclonal 5mC antibodies to bind to the methylated DNA. After this occurs, magnetic beads each containing a secondary antibody with high affinity for the primary antibody will be added to the sample, which will be incubated once again. These bead-linked antibodies will bind to the monoclonal antibodies. Then, DNA bound to the antibody complex (methylated DNA) will be isolated from the rest of the DNA with the use of a magnet that separates the antibody complexes from the solution. Following this, several washes will be performed using an IP buffer to remove all the unbound, non-methylated DNA in the sample. An enzyme known as Proteinase K will then digest all the antibodies, leaving only the methylated DNA inside the sample. Finally, the enriched DNA will be purified by a technique known as phenol:chloroform extraction to remove the protein matter inside the sample and will then be precipitated in water to be used later. After MeDIP is performed, PCR will be performed to obtain data of the precise methylation levels across a wide genomic range. This data will be compared before and after the hypomethylating agents are applied to determine precisely how much of the methylation was removed from H3K4me1 marks of the VHL tumor suppressor genes by the hypomethylating agents and at what precise location these demethylation events occurred. This is crucial data that will allow researchers to determine on which CpG enhancer/promoter these agents act on to demethylate hypermethylated tumor suppressor genes in renal cells.

Use of hypomethylating agents in the downregulation of overactive Wnt Pathways by reactivation of the Wnt antagonist, WIF-1.

Hypomethylating agents will be directed to reduce the activity of the Wnt pathway in ccRCC by reactivation of a particular Wnt antagonist. The Wnt pathway consists of a set of crucial signal transduction pathways that work together to regulate cell growth and proliferation, migration, cell fate, among other things. In cancer, however, the Wnt pathways go into overdrive. Meanwhile, Wnt inhibitory factor-1 (WIF-1) is downregulated by promoter methylation, which hinders it from performing its main function of suppressing the activity of molecular players in the Wnt pathway when they overact. The overactive Wnt pathways result in aberrant cell growth and proliferation, migration, avoidance of apoptosis, among other things – which are all characteristic of cancer cells. Methylation of WIF-1 is therefore a key factor that contributes to the development and progression of ccRCC. Hypomethylating agents, however, can offer a solution to this. These agents can work to demethylate WIF-1 to reactivate its inhibitory activity in order to reduce the activity of the overactive Wnt pathways. Therefore, they can inhibit the proliferation and migration of many RCC cell lines, hindering the progression of the carcinoma.

Use of RT-qPCR to determine the efficacy of hypomethylating agents in reducing gene expression by downregulation of the Wnt Pathways.

To examine whether the hypomethylating agents were effective in reducing the activity of the Wnt pathways by the activation of WIF-1, reverse transcription quantitative polymerase chain reaction (RT-qPCR) will be performed. First, mRNA will be extracted from the genes that are affected by WIF-1 – namely, those responsible for cell growth and proliferation, cell migration, and apoptosis – and the enzyme reverse transcriptase performs reverse transcription on the mRNA, converting it into cDNA. The cDNA (which is single-stranded) is then converted into double-stranded DNA (dsDNA) by DNA polymerase II, which adds primers to specific regions of the cDNA and creates a complementary strand. Following this step, the reverse transcriptase enzyme is deactivated and the qPCR process begins for amplification of the RNA. In this process, the dsDNA is denatured by heat (95°C) and separated into two single strands. Next, β-actin primers will bind to the single DNA strands during annealing at a temperature of no lower than 40°C. In the final step, elongation, the primers are further extended by DNA polymerase II, which ultimately results in two copies of the original DNA strand. Finally, these strands will be analyzed with a graph displaying fluorescence intensity corresponding to mRNA expression. RT-qPCR will be performed to compare mRNA expression levels before and after the administration of hypomethylating agents in order to determine the efficacy of the hypomethylating agents in reducing the activity of overactive genes influenced by the hyperactivity of the Wnt pathways. The mRNA levels, and thus the level of expression of the genes affected by WIF-1 will also be compared to the β-actin housekeeping gene, which is the internal control of the RT-qPCR test. With this graphical analysis, it is possible to determine whether the hypomethylating agents could be used as plausible molecular players in downregulating overactive cancer-promoting Wnt pathways.

Tips!

- Be transparent about how you will conduct each experiment and how you'll measure your results
- Be realistic about what's achievable
- Address any potential concerns preemptively

Citations...

1. *Support your claims*

2. *Demonstrate familiarity*

3. *Build credibility*

1. *Forgetting to cite a key study in your field*

2. *Using outdated sources for cutting-edge fields*

3. *Mismatched references*

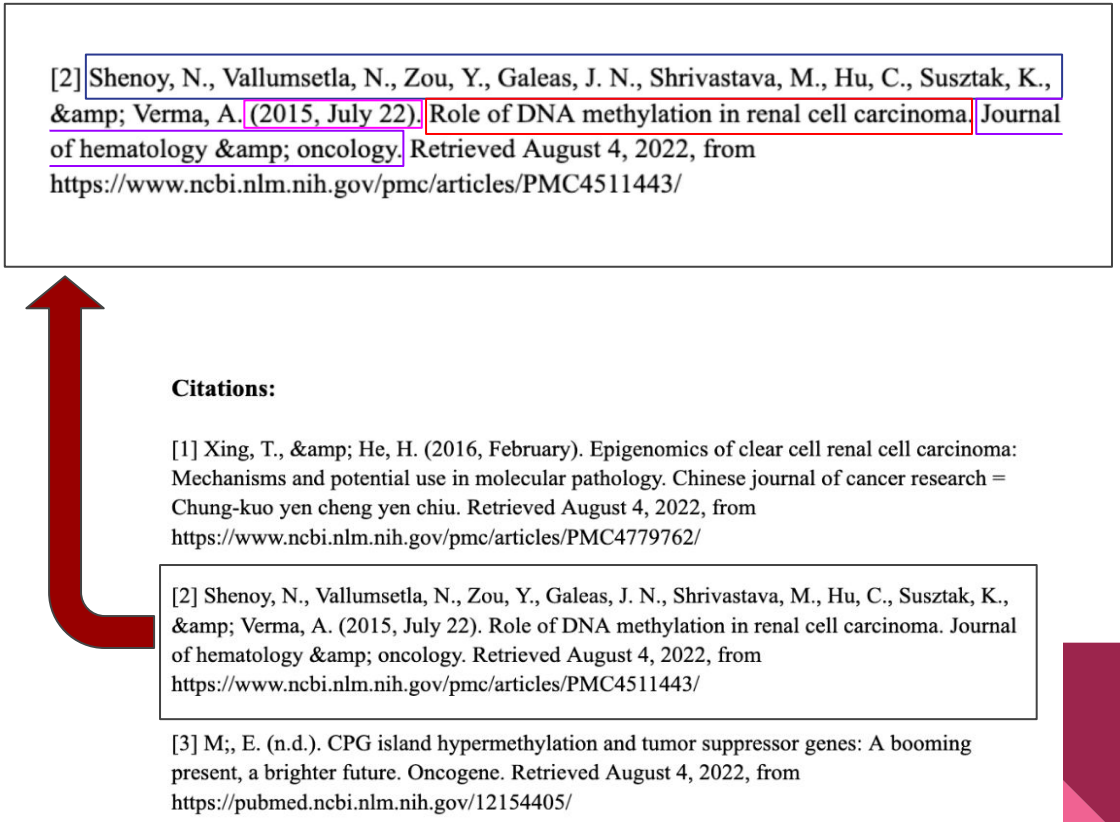
How to Format Citations

- Numbered citation format: **Vancouver-style**

- Include author names, title of the article, journal name, publication date, and volume

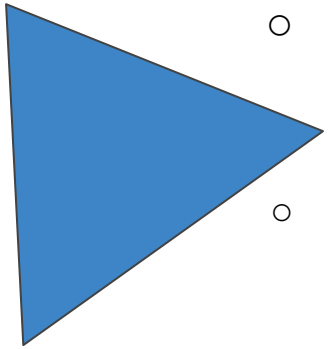
- Ensure the relevance of the sources

- Maintain consistency!



Crafting a Persuasive Proposal

- Be clear, concise, and compelling
  - Avoid jargon
  - Stay within page limits; avoid redundancy
  - Emphasize your project's significance



- Write measurable hypotheses and objectives
  - SMART
    - "To evaluate the efficacy of drug X in alleviating pain severity by 40% over 6 months."
  - Testable hypotheses
    - "We hypothesize that [variable] will [effect] on [outcome] through [mechanism]."

- Tailor to the funding institution
  - Align with institution;s mission
  - Address their stated goals with their language

Budget and Justification

- **Key components of a budget**
  a. Personnel
  b. Equipment
  c. Materials
  d. Travel
  e. Indirect costs

- **Writing a persuasive budget justification**
  a. Link expenses to specific project aims
  b. Provide detailed rationale for each item that's needed
  c. Emphasize cost effectiveness & alignment with the funder's policies

- **Common mistakes and how to avoid them**
  - Over/underestimating costs
  - Including unnecessary or inappropriate items
  - Misaligning budget with your proposal narrative

Final Tips for Grant Writing Success

1. Start early

2. Understand your audience

NIH
National Institutes of Health

World Health Organization

NHS

3. Be clear and well-organized

4. Seek feedback

5. Prepare for resubmission

Thank you for listening!

Now it’s time for questions :)