PROTACs: Catalyzing MEK4 Degradation to Improve the Efficiency of Cancer Treatment

SURG | Natural Sciences and Engineering (NSE) | *Tags: Lab-based*

*This cover page is meant to focus your reading of the sample proposal, summarizing important aspects of proposal writing that the author did well or could have improved. **Review the following sections before reading the sample**. The proposal is also annotated throughout to highlight key elements of the proposal's structure and content.*

Proposal Strengths	Areas for Improvement
New concepts are presented one sentence at a time, and jargon is defined in simple terms for a broad audience throughout.	While an aim/objective statement is present, rephrasing to create or including an explicit research question could help add clarity.
Researcher clearly articulates parameters of experiment and how they are going to validate their work.	
While you may be working with others in a lab setting, this student highlights their independent contribution to the overall project by using "I" language.	
Methods are structured in a way that makes it clear this project is feasible within 8 weeks.	

Other Key Features to Take Note Of

While figures are not mandatory, they can be included in an appendix. If you choose to include a figure or preliminary results, please see the "Drafting Appendices" under "Developing Your Application Package" on the OUR website. This particular student uses simple schematics to explain the complex jargon in their proposal.

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Cancer occurs when cells undergo uncontrolled replication and spread throughout the body. Currently, one of the most lethal cancers is pancreatic cancer. It has been shown that genetically removing overactive proteins, like MEK4, sensitizes pancreatic cancer cells to an FDA-approved drug that targets MEK1/2, another protein in the same protein family. I aim to chemically remove MEK4 from cells by developing a new molecule that exploits the natural protein degradation machinery of the cell to remove the target protein. The development of a chemical tool through the combination of a Scheidt group MEK4 inhibitor and a protein-degrading catalyst should improve the efficiency of existing cancer treatments.

By 2030, pancreatic cancer is predicted to be one of the top three most lethal cancers.[1] Cancer occurs when healthy cells have mutated proteins which allow uncontrolled cell division. These cells multiply rapidly due to unregulated cellular signaling pathways. Frequently, in pancreatic and other cancers, the mitogen-activated protein kinase (MAPK) pathway, a pathway present in most organisms that communicates a signal from a cell surface receptor to the DNA in the nucleus of the cell, is over-activated, permitting tumor cells to avoid cell death.[2] Notably, these cancers often have overactive MEK4, a protein in the MAPK pathway, due to genetic mutations.[3] A study by Xue *et al.* showed that genetic removal of MEK4 sensitizes the cancer cell to inhibitors that disable other proteins in the pathway, such as MEK1/2.[2] Treatment of MEK4-absent tumor cells with selumetinib, an FDA-approved MEK1/2 inhibitor, resulted in complete cell death. However, this drug treatment was ineffective against unaltered tumor cells. Slowing the growth of cancerous tumors and preventing spread throughout the body is essential for treatment. Therefore, I want to investigate potential novel co-treatment of MEK1/2 and MEK4 inhibitors.

Although editing the genome of a cell in a laboratory to produce a mutation is useful for research, it is not currently feasible for treating a cancerous tumor in the body. Therefore, I aim to reproduce the results of Xue *et al*. through chemical treatment of MEK4. This summer, I will go a step beyond chemical inhibition by developing a molecule that causes "chemical knockouts," where the target protein is not only inhibited, but also degraded within the cell. **Pro**teolysis-**Ta**rgeting **C**himeras (PROTACs) are a new category of small molecule inhibitors that can destroy proteins within a cell without genetic manipulation of the entire organism, ensuring minimal side effects beyond the desired degradation.[4] PROTACs function by exploiting the ubiquitin-proteasome pathway, the natural mechanism that cells use to digest old or malfunctioning proteins. First, a ubiquitin protein chain is attached to the target protein using a ubiquitin ligase (E3) enzyme. Then, the ubiquitin-labeled protein is further ubiquitinated, recognized, and degraded by the proteasome, a cellular machine that digests proteins into amino acids.[4] Due to their small size, PROTACs are highly cell-permeable, allowing them to easily reach the target of interest.

PROTACs consist of a molecule that binds to the target protein, a molecular tag that attracts the E3 enzyme, and a linker that connects the two molecules (**Figure 1**).[5] PROTACs require target-selective molecules in order to achieve high specificity for the target protein. I will synthesize my PROTACs by building off of a potent MEK4 inhibitor developed by the Scheidt lab. Several combinations of linkers and E3 enzyme tags are commercially available. The length of the linker is important as it determines if the E3 enzyme tag will bring the E3 enzyme close enough to the target protein to catalyze ubiquitin labeling (**Figure 2**). A spacer that is 12 atoms long has been shown to be flexible enough to accommodate the E3 enzyme while remaining functional.[4] By selecting an E3 enzyme tag and varying the length and composition of the linker, I will optimize a PROTAC that leads to the degradation of MEK4.

Throughout the winter and spring quarters, my research is focusing on the synthesis of molecular analogs of an effective MEK4 protein inhibitor. Thus far, I have completed the synthesis

Margin annotations:
- The focus of the project is clear within the first paragraph.
- Background draws on work from other relevant fields with clear logic flow.
- Justifies approach of the project.
- While there is an aim statement, those aims could be strengthened by including an explicit research question.
- Identifies gap in knowledge.
- Student uses " I" language throughout as a way to emphasize independent contribution.
- Research question/goal is clearly stated.

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of three analogous molecules and will evaluate their potency using *in vitro* assays. This summer, I will build PROTACs using the original MEK4 inhibitor developed by the Scheidt group, unless my research yields a more potent molecule, in which case I will use the more effective compound. This inhibitor will allow the PROTAC to selectively target the MEK4 protein as it exploits the cellular degradation machinery.

To develop the PROTAC, a linker molecule and tag will be attached to the MEK4 inhibitor. Various combinations of linkers and tags are commercially available. PROTACs tested on pancreatic cancer cells have featured thalidomide as a molecular tag to attract the E3 enzyme.[5] Therefore, I will begin my research by focusing on linkers with thalidomide tags. The composition of the linker affects the solubility as well as the flexibility of the PROTAC. To optimize the best length and composition of linker, I will test six types of linkers: three PEG (oxygen-containing) and three carbon chain linkers. The linkers that are commercially available will have a minimum length of 12 atoms. I will use an amide coupling, a robust chemical reaction, to attach the linker to the inhibitor molecule. Mass spectrometry will be used to confirm that the final product has the expected molecular weight. The chemical structure of the PROTAC will be confirmed using Nuclear Magnetic Resonance (NMR), a technique that identifies hydrogen and carbon atoms through graphical patterns that indicate the locations of the atoms in the molecule.

Once the six PROTACs have been developed, their potency can be evaluated using a Western blot, a technique used to identify and quantify the amount of protein present within a cell. Specifically, I will apply each PROTAC to Human Embryonic Kidney (293-HEK) cells, a standard choice of cell line to test potency. The cells will be incubated with PROTAC so that the drug can catalyze the degradation of the MEK4 protein. Once the incubation period is complete, the cells will be ruptured and heated in order to denature, or unfold, every protein. The unfolded proteins can then be loaded into a polyacrylamide gel. When a current is applied, the negatively charged proteins will travel vertically down the gel plate; the smaller proteins are able to move more quickly through the gel, separating by size. The proteins will be transferred onto a membrane and treated with a primary antibody that specifically binds to MEK4. As a control, a different primary antibody will also be used to mark a "housekeeping" protein with constant cellular levels which can be used to normalize the MEK4 bands of interest. A secondary antibody with a fluorescent tag will then be added to label the MEK4 band and the "housekeeping" band, respectively with different colors. The fluorescent intensity is directly related to the quantity of protein within each cell. Therefore, the less fluorescent bands contain fewer proteins, and the most effective PROTAC will yield the least fluorescent band after normalization. Comparing the results of the Western blot will allow for visualization of the effectiveness of each PROTAC in degrading the MEK4 protein within the human cell. Although beyond the scope of this proposal, I ultimately hope to identify highly potent PROTACs and test them in cellular and *in vivo* mouse models of pancreatic cancer.

At the start of the summer, I will have spent two quarters working with the Scheidt group. I am experienced in synthesizing the starting MEK4 inhibitor used as the base of the PROTAC– I have already synthesized three analogs and am hoping to complete three more by the summer. I have experience using NMR spectroscopy to analyze the structure and purity of a final product. The graduate student serving as my mentor will train me to perform Western blots, in addition to providing guidance in chemical synthesis. This research synthesizes knowledge and techniques from courses I have taken such as Organic Chemistry, Chem 220 (Introductory Instrumental Analysis), and Cell Biology. As I prepare for medical school, this work will help me think critically about how knowledge of existing cellular processes can inform the development of drugs using medicinal organic chemistry in order to improve survival rates for lethal cancers.

Methods are defined and explicitly justified in terms of how each step helps to answer the research questions.

Researcher lists preparation using relevant courses and previous experiences. They connect it back to their personal goals.

Clearly states the variable to be tested as well as the scope of the testing.

Student explains how their work will be validated.

Clearly states controls in experimental approach.

Articulates parameters of success.

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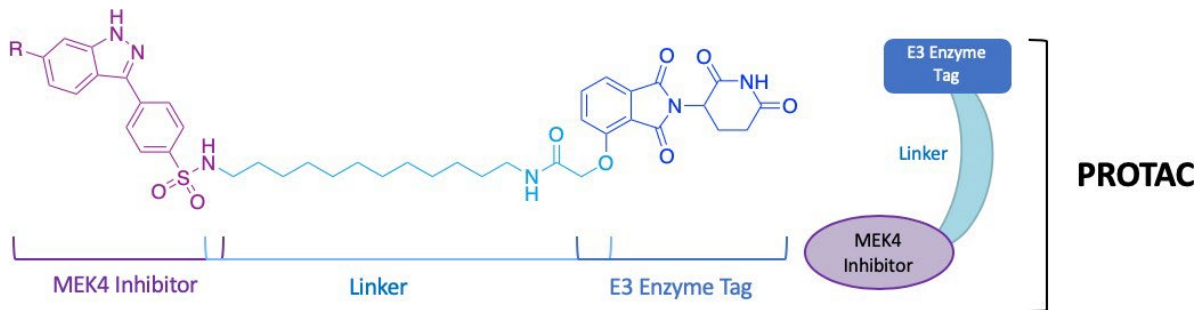
Figures

Figure 1: Molecular structure of a PROTAC containing a MEK4 inhibitor and E3 enzyme tag connected by a linker. My current research focuses on analyzing the potency of analogs of the MEK4 inhibitor by changing the structure of the 'R' group on the far-left side of the chemical structure.
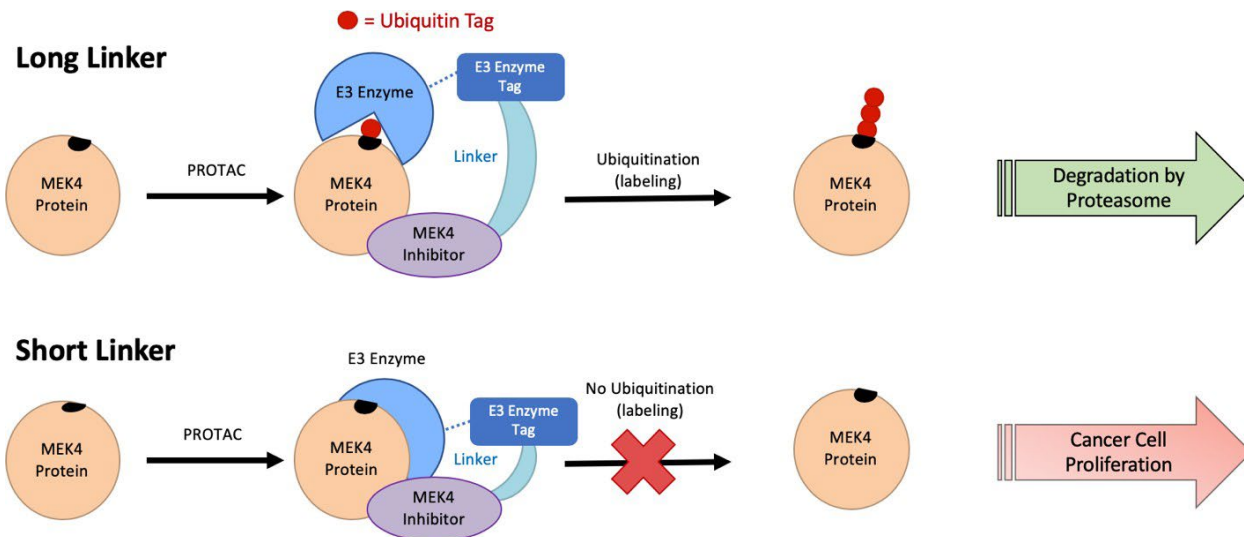

Figure 2: Scheme demonstrating the catalytic ubiquitin labeling of MEK4 by PROTACs. The importance of the linker's length and flexibility is emphasized in this visual representation; if the E3 enzyme tag is unable to reach the ubiquitination site of MEK4, the E3 enzyme will not be able to label MEK4.

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References
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(2) Xue, Z.; Vis, D. J.; Bruna, A.; Sustic, T.; van Wageningen, S.; Batra, A. S.; Rueda, O. M.;Bosdriesz, E.; Caldas, C.; Wessels, L. F. A.; Bernards, R. MAP3K1 and MAP2K4 Mutations Are Associated with Sensitivity to MEK Inhibitors in Multiple Cancer Models.*Cell Research* **2018**, *28* (7), 719–729.
(3) Cunningham, S. C.; Gallmeier, E.; Hucl, T.; Dezentje, D. A.; Calhoun, E. S.; Falco, G.; Abdelmohsen, K.; Gorospe, M.; Kern, S. E. Targeted Deletion of MKK4 in Cancer Cells: ADetrimental Phenotype Manifests as Decreased Experimental Metastasis and Suggests a Counterweight to the Evolution of Tumor-Suppressor Loss. *Cancer Res* **2006**, *66* (11), 5560–5564.
(4) Schneekloth, John S.; Fonseca, F. N.; Koldobskiy, M.; Mandal, A.; Deshaies, R.; Sakamoto, K.; Crews, C. M. Chemical Genetic Control of Protein Levels: Selective inVivo Targeted Degradation. *J. Am. Chem. Soc.* **2004**, *126* (12), 3748–3754.
(5) An, S.; Fu, L. Small-Molecule PROTACs: An Emerging and Promising Approach for theDevelopment of Targeted T

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