GRANT APPLICATION FORM

Check the Grant Award you are seeking.

_____Individual Development/Course Development Grant
___X___Critchfield/Cornell Research Grant
_____Ashforth Research Grant

Applicant Information:

Principal Researcher: Kasandra Riley
Academic Rank: Assistant Professor
Department: Chemistry
Office Phone Number: 407.646.2250
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Grant Proposal:

Short Title of Grant Proposal: Exploring the Role of EBV microRNAs in Apoptosis
Proposed Start Date: September 1, 2013
Proposed End Date: May 31, 2014

Description of Grant Proposal:

Objectives of grant proposal:

Please list objectives that are clear, specific, concrete, and measurable.

The virus-host relationship is an epic battle between the immune system and viral escape. A classic example is the interplay between human B cells and Epstein-Barr virus (EBV). EBV infects >95% of humans for life, expressing few genes in the genetic program "latency." Sometimes, healthy B cells convert from quiet, latent EBV infection to become lymphomas (B cell cancers). Precisely how the virus causes cancer or how to eliminate EBV-induced malignancies is unknown.

MicroRNAs (miRNAs) are ~22-nucleotide-long non-coding RNA genes that regulate messenger RNA (mRNA) target gene transcripts through base-pairing (binding) interactions with partially complementary sequences. MiRNAs direct the repression of gene transcripts involved in diverse cellular processes. Humans express >1,000 miRNAs, and EBV expresses at least 49. Each miRNA regulates hundreds of target mRNAs, and each mRNA has multiple miRNA regulators. The function of the EBV miRNAs is not well understood, but they functionally contribute to the cancer phenotype of the virus.

One of the hallmarks of cancer is the evasion of "apoptosis," the normal cellular capability to self-destruct when the cell becomes deranged. When apoptosis is inhibited, sick cells can continue to proliferate unchecked, leading to cancer. EBV is known to deregulate apoptosis during cellular transformation, and my previously published data from large-scale screens demonstrated that EBV microRNAs might target many genes directly involved in apoptosis. *This project seeks to move beyond the initial genetic screen to verify and validate specific gene targets of the virus that may be involved in cancer transformation and maintenance.*

For the first year of this project, my goal is to study two target genes, central apoptosis regulator caspace 3 and a second target of the same miRNAs that target caspace 3, which will be confirmed after bioinformatic analyses of related genes at the outset of the project. The approach below will be employed for both targets in parallel, as it represents the current standard in methodology for validation of miRNA targets:

1. Confirm the direct repression of targets by luciferase assay. Using basic molecular biology methods, portions of the target genes of interest will be introduced into testable plasmid vectors along with a "reporter gene" that, when expressed in human cells, lights up in an assay in response to the amount of gene that is turned on. These vectors, along with synthetic miRNA mimics or inhibitors, will be co-introduced (transfected) into human cells, and the regulation of the gene will be measured by a light assay. Gene transcripts that are regulated by a given miRNA will emit more light in the absence of that miRNA than when its presence.

2. Confirm absence of repression of mutant targets by luciferase assay. For the gene targets that pass the first test for direct repression in the luciferase assay, the area of regulation within the gene will be mutated by site-directed mutagenesis, and this mutant reporter will be tested as above. If the target passes this second test, it will not be impacted by the addition of miRNA mimics or inhibitors.

3. Confirm the repression of targets at the protein level. The luciferase assay is necessary to test for the direct repression of target transcripts, but it does not inform about the effect of the miRNAs on the natural target protein levels. Protein levels are tested by transfecting human cells with miRNA mimics or inhibitors and subjecting the cells to Western blot analysis. Specific antibodies, which will bind to the protein of interest, will be able to detect the different amounts of protein in the differently treated cells. Presumably, the miRNA mimic will decrease protein levels, and the miRNA inhibitor will enhance them relative to an untreated control.

When examined collectively, the experimental results from these three objectives will confirm the existence of novel gene transcript targets of miRNAs. There are over 100 different apoptosis-associated putative targets of EBV microRNAs for students to pick from when designing their experiments, and such experiments have been highly successful for my previously mentored undergraduates. The validation of each miRNA target provides an improved understanding of the mechanism of this virus when it infects human cells.

Describe the expected outcome(s) for this project (publication, performance, exhibit, paper).

This original work will be publishable in a peer-reviewed journal such as *RNA*, *Biochemical and Biophysical Research Communications,* or the new journal *microRNA.* Regardless of the experimental outcome, this project can also be presented as a poster or oral talk at a research conference such as the American Society for Biochemistry and Molecular Biology (ASBMB) Annual Meeting or the Annual Meeting of the RNA Society in 2014.

Describe how this project relates to your current expertise.

My Ph.D. research explored the role of a tumor-suppressor protein. My major research project as a postdoctoral research fellow during the past five years culminated in the publication of the first comprehensive list of the possible gene targets of Epstein-Barr viral microRNAs, which is the preliminary data upon which this grant is based. This project exemplifies my area of expertise. I have previously executed and published experiments employing all of the proposed techniques.

Describe the relationship of this project to your long-term professional goals.

This project will be my first as a fully independent researcher. Having just exited the fast-paced world of R1 biomedical research, I am striving to keep in touch with this type of research by executing smaller-scale, cutting-edge work in my newly established laboratory at Rollins. In time, the work undertaken will afford me opportunities to present my work as I network with colleagues in my discipline and contribute to my efforts to obtain tenure at Rollins.

Describe the contribution this project makes to your field and to the academic community.

While thousands have been proposed in large-scale screening experiments, very few direct targets of EBV microRNAs have actually been validated in the literature. There is a high false-positive rate in the large-scale experiments, making such studies crucial to our legitimate and complete understanding of the function of these viral genes in cancer. Thus, such studies are highly publishable and relevant. It will contribute to the understanding of both EBV biology and the development or maintenance of virally induced cancer. It was my first mentored undergraduate research experience that led me to my

present career. Rollins students who work with me will learn firsthand about the challenges and excitement of biomedical research and they gain important experience, and I hope to inspire the next generation of cancer researchers.

Describe the contribution this project will make to Rollins College.

Chiefly, this project will afford the opportunity for several students to gain hands-on, independent biomedical research. This project is interdisciplinary, and students will gain skills in bioinformatics, statistics, molecular biology, and human cell culture, in addition to widely applicable abilities in constructing proposals, testing hypotheses, and experimental planning/organization outside of the classroom. Further, undergraduates must accomplish at least one major research project to be competitive applicants to medical and graduate school, and most of the Rollins students majoring in biochemistry & molecular biology enter into these programs. Because my research projects are biomedical in nature, this will be of particular relevance. Finally as a new faculty member, I am introducing a new field of research to Rollins: cancer virology.

Proposed Budget:

Please review Permitted Expenditures section and provide as much detail as possible. Be specific about what costs will be incurred for travel, telephone, staff support, photocopying, etc. Please provide sources for estimates where available (e.g. international per diem rates listed on the U.S. Dept. of State webpage or flight costs posted online and date consulted). Please include as specific as possible details and estimates for travel (dates, termini, park or museum entrance fees, car rentals). If your proposal requests funds for a student assistant, please specify the skills and tasks related to the student worker as well as the anticipated number of work hours and hourly wage. This budget will be for one year only. Your proposed budget should reflect your actual anticipated permitted expenditures for the project, even if this figure exceeds the allowed award maximum of $5000. Please also reflect other sources of revenue.

Item	Justification	Amount
Equipment/Supplies	*Consumables* Caspace 3 antibody: ($235) Tubulin antibody: ($293) Anti-rabbit secondary antibody: ($128) ECL reagent for visualizing Western blots: ($182) Cell culture media and additives: ($350) FBS for cell culture, 2x500 ml: ($498) Supply chemicals (NaCl, Tris, EDTA, etc.): ($606) Cell transfection chemicals, Lipofectamine 2000, 5 ml: ($510) Nitrocellulose membranes: ($151) Latex gloves, 10 boxes: ($122) Eppendorf (1.5 ml) test tubes, 500: ($235) Pfu Turbo DNA polymerase, 500 units: ($458) Deoxyribonucleotide triphosphates (dATP, dGTP, dCTP, dTTP): ($628) Shipping costs: ($100)	$ 4496
Personnel Support		$ 0
Travel		$ 0
Other		$ 0
Total Anticipated Budget Expenditures	(May exceed $5000)	$ 4496
TOTAL Requested Funds	(May not exceed $5000)	$ 4496

Previous Funding from Rollins College:

1. Have you received funding within the last 10 years for a Critchfield/Ashforth Research, Individual Development, Course Development, or Cornell Research Grant? **YES** **NO**

If possible, please forward previous final reports to Karla Knight, kknight@rollins.edu.
If yes, list all grants received:

2. Have you applied for other grants from Rollins this year? **YES** **NO**
   If yes, list all grants applied for, grants awarded, and amounts to be received:

3. Have you received any other grants or funding from Rollins during the previous five years? **YES** **NO**
   If yes, list all grants awarded and amounts received over the last five years:

   I was granted new faculty start-up funds, $57,000 for capital equipment (see below) to be used prior to August 2015.

Other Support for Current Proposal

1. Have you applied for or been granted any external or other internal sources of funding for this proposal? **YES** **NO**

   If yes, clearly identify all other requests that duplicate this proposal, indicating the periods and amounts of all support requested and/or received, along with the status of the support.

   If you are requesting funds for a second or third year of support for one project, attach a progress report.

I have been granted start-up funding to be used through my third year as part of my hiring package from Rollins to pay for capital equipment necessary for initiating my laboratory from scratch. In the present request, I am asking to fund non-duplicate consumables to be used in my first year of research, which are NOT covered by the start-up funding.

Attach to this application:

Abbreviated Vita (3 pages maximum) listing all papers published and/or presented that are related to this project. If this is a new area for you, list your most recent publications/ presentations. (attached)

Department Approval Statement: A statement endorsing the project from the department chair, director, or appropriate dean is required for all proposals where the proposed outcome may impact departmental curriculum or require departmental resources (budget, supplies, space, or personnel.) (N/A)

Research Review Board Approval: If the proposed research involves human subjects or vertebrates, a letter of approval from the requisite board should be attached to this grant application (IRB for human subjects or IACUC for vertebrates). (N/A)

Signature of Principal Researcher  **Date** __1.12.10

Send this application and your vita electronically to the Dean of the Faculty Office via Karla Knight, kknight@rollins.edu. If possible, please submit both files as PDFs.