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| **Applications will be accepted from:** [x]  Both MD & non-MD Undergraduates [ ]  MD undergraduate students only [ ]  non-MD undergraduate students only |
| **Project Duration:** [ ]  Suitable for either a 4 or 8 week project (Only Yr 3 MD students are eligible to apply for 4-week projects) [x]  Only suitable for an 8-week project [ ]  Only suitable for a 4-week project  |
| **Additional information for potential student partners:** E.g. desired skills/interests/experience, scheduling restrictions for the project timeline, additional info you want applicants to provide when contacting you about this position, etc. **Student in third year or above is preferred**      |

## PROJECT INFORMATION

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| **Project Title:**      **Role of transcription factor NFAT5 in coxsackieviral pathogenesis of viral myocaditis** |
| **Hypothesis or Research Question being addressed (400 character limit, ~55 words):**We previously found that NFAT5 is an antiviral transcription factor but is cleaved at Glycine503 by coxsackieviral proteases and inactivated. To explore the potential of NFAT5 as an antiviral agent, we ***hypothesize*** that CRISPR/Cas9-mediated gene editing can generate a stable cell line expressing an uncleavable NFAT5G503A mutant protein (changing Glycine503 to Alanine), which will resist coxsackievirus infection and reduce viral pathogenesis. |
| **Keywords:** **Provide approximately 5 key words that describe the proposed research project.**Myocarditis, coxsackievirus, pathogenesis, CRISPR/Cas9, NFAT5, antiviral |

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| **Project Attributes and Benefit to the Student**Please review the [online adjudication criteria](http://www.med.ubc.ca/current-learners/summer-student-research-program/adjudication/) carefully prior to completing the next two sections to ensure your application is addressing the adjudication criteria outlined in “Project Attributes and Benefits to the Student”. |
| **A) Background and Summary of Proposed Research. Summarize the proposed project including the rationale for the project, the context within the relevant field of research, the proposed research approach and the expected project outcomes.** *If this is an ongoing project of >8 weeks duration (or 4 weeks for MD 2022 students) clearly distinguish the expected project outcomes at the end of the FoM SSRP funding period from the overall project objectives.* **Please write in lay terms for a non-specialist audience.** **Character Limit: 3050 characters (~430 words)**Heart disease is the leading cause of death worldwide. Viral myocarditis is an inflammatory heart disease caused by viral infection, particularly in children and young adults. In patients less than 40 years of age, this infection is a major natural cause of sudden death, accounting for approximately 20% of all such cases. To date, there is no specific therapy or vaccine for this disease. Among different viruses, Coxsackievirus B3 (CVB3) is the most common causal agent of viral myocarditis.CVB3 proteases play an important role in viral pathogenesis by cleaving cellular proteins. Nuclear factor of activated T-cells 5 (NFAT5) is a transcriptional factor activated during cellular immune response. Interestingly, our previous studies showed that NFAT5 protein was cleaved at Glycine 503 (G503) by CVB3 protease, and subsequently inactivated. We also found that overexpression of wild-type (WT)/full length NFAT5 by plasmid strongly inhibited CVB3 replication, indicating a therapeutic potential of NFAT5 in anti-CVB3 treatment. However, the overexpressed WT NFAT5 will still be cleaved by CVB3 protease during infection, which impairs the anti-viral activity. To further confirm the antiviral property of NFAT5 and explore the potential of NFAT5 as an antiviral agent, we propose to generate a new cell-line by CRISPR/Cas9-mediated genome editing to express an uncleavable NFAT5G503A (changing Glycine503 to Alanine) protein, which may achieve a strong anti-CVB3 effect. We hypothesize that expression of NFAT5G503A in host cells will inhibit CVB3 replication and virus-induced pathogenesis.Aim 1. To generate a mutant plasmid expressing the uncleavable NFAT5G503A by PCR-mediatedmutagenesis and test its antiviral activity by transient transfection. The mutant plasmid expressing NFAT5G503A will be constructed by PCR and transfected into CVB3-infected cardiomyocytes. Cell death will be evaluated by MTS cell viability assay. Meanwhile, CVB3 replication will be evaluated by Western blot to detect viral protein VP1 and plaque assay to detect viral particle formation.Aim 2. To generate a stable cell line expressing the uncleavable NFAT5G503A by CRISPR/Cas9-mediated gene editing. The coding DNA fragment of a single guide RNA (sgRNA) targeting NFAT5 will be synthesized and cloned into a vector expressing Cas-9. Meanwhile, a single-strand oligo DNA nucleotide (ssODN) harboring NFAT5 G503A mutation site and two flanking arms base-pairing with the adjacent sequences will be synthesized. The plasmid and the ssODN will be co-transfected into CVB3-infected cells and the stable cell line will be identified by sequencing. The antiviral activity will be evaluated in the same way as mentioned above.**Expected Results**:This is a part of an ongoing CIHR-funded project. We have constructed the mutant plasmid and expected that the transient transfection of plasmid NFAT5G503A into cells will suppress CVB3 replication, which will depend on the transfection efficiency. However, stable NFAT5G503A cell line produced by CRISPR/Cas9 will sustainably resist CVB3 replication*.* |
| **B) Outline the student’s role in the project and describe how they will benefit from their involvement.** This section must address how involvement in this project will help the student gain an understanding of how high quality research is conducted. This includes addressing the opportunities to learn new skills in the context of the relevant learning objectives listed in the [adjudication criteria](http://www.med.ubc.ca/current-learners/summer-student-research-program/adjudication/); their anticipated interactions with other researchers and the available resources that will contribute to a beneficial experience.*Clearly indicate which items will be completed during the FoM SSRP funding period and which (if applicable) will be completed before or after the funding period if the student and supervisor have chosen to also work together outside of the funding period. Project feasibility is considered during the adjudication process; 4-week and 8-week projects will be adjudicated separately, with appropriate consideration given to each.***Character Limit: 3800 characters (~540 words)**This is an ongoing project supported by a CIHR grant. Since most of the techniques mentioned above have been established in our laboratory, the student will mainly learn and perform some of the basic experiments. For Aim 1, the student will i) conduct PCR to generate the designed mutations on NFAT5 gene, ii) perform molecular cloning of the PCR-generated DNA fragments containing the mutation and iii) identify the clones by restriction digestion or PCR. For Aim 2, the student will be involved in i) the design of the single guide RNA (sgRNA) and the single-strand oligo DNA nucleotide (ssODN) harboring NFAT5 G503A mutation site, ii) co-transfection of cardiomyocytes to produce PRISPR/Cas9-mediated mutation. The student will also participate in certain more advanced experiments such as identifying the stable mutant cell lines by fluorescence-activated cell sorting (FACS), Western blot analysis of the viral capsid protein and MTS assay to determine the cell viability after infection of the NFAT5G503A mutant cells. The student will learn these new skills during his/her participation of the research work. The student will work on this project under the direct supervision of a PhD student and a Postdoctoral Research Associate who work on the same project. All the facilities for this study are available for this student. In addition, the student will also participate in the discussion of other related projects (i.e. molecular pathogenesis of coxsackievirus and signal transduction pathways in viral myocarditis) during the weekly laboratory meetings and the monthly journal club to learn more knowledge in cardiovascular research. The student will also attend the weekly RIP (Research in Progress seminar) in our Research Center and will give an opportunity to present a 15-min RIP in the summer. At the end of the summer, a Summer Student Research Day will be held in our hospital, the student will either present a poster or a 10-min oral presentation based on his/her research results. This event will enable the student to learn how to critically analyze and present the research data to communicate to other researchers.We expected that we will finish the Aim 1 and most parts of Aim 2 by the end of the summer. Theremaining experiments will be continued in the fall. There is a possibility that the student will be continuously involved in this project outside of the funding period. For this study, we will get preliminary data to verify that the uncleavable NFAT5 is a potent anti-CVB3 protein and worth further testing in an animal model. |

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| **Please indicate if your project requires the following and indicate their status as appropriate.** This will help clarify the scope of the project for potential student partners. |
| **This project requires ethics approval (human or animal):**[x]  Yes [ ]  NoIf yes please indicate if you:[x]  Already have approval [ ]  Will obtain approval before the SSRP funding period [ ]  Intend for ethics application to be a focus over the funding period\*Please note that as ethics approval can be a lengthy process it is recommended that this be obtained well in advance of the funding period unless the intention is for this activity to form a major part of the FoM SSRP-funded portion of the project.**This project requires access to electronic medical records:**[ ]  Yes [x]  NoIf yes please indicate if you: [ ]  Already have approval [ ]  Will obtain approval before the SSRP funding period [ ]  Plan to obtain approval during the SSRP funding period**This project requires operational/institutional approval:**[x]  Yes [ ]  NoIf yes please indicate if you: [x]  Already have approval [ ]  Will obtain approval before the SSRP funding period [ ]  Plan to obtain approval during the SSRP funding period |

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| **Research Location (As applicable, indicate where the project will be conducted.)** |
| City or Region: VancouverResearch Centre: Center for Heart and Lung InnovationHospital:      St. Paul’s HospitalProgram or Unit:      Additional information (building, lab etc.):       |
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| **Supervisor’s Information** |
| **Supervisor Last Name:**      Yang | **Supervisor First Name:**      Decheng |
| **FoM Department/School (Main FoM Appointment):**      Pathology and Lab Medicine | **UBC FoM Division (if applicable):**      |
| **Preferred contact method (for students)****[ ]** Phone supervisor**[x]** Email supervisor | **[ ]** Phone alternate contact**[ ]** Email alternate contact |
| **Preferred Phone:**       | **Supervisor Rank (Instructor, Professor etc.):**     Professor |
| **E-mail Address:**       decheng.yang@hli.ubc.ca |  |
| **Optional Alternate Contact** (e.g. co-supervisor, research/lab coordinator, assistant etc.) |
| **Contact’s Name:**       | **Contact’s Role:**       |
| **Contact’s Phone Number:**      | **Contact’s E-mail Address:**      |