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**TITLE:** Role of lncRNA-Encoded Micropeptides in Cholangiocarcinoma

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14. ABSTRACT Cholangiocarcinoma (CCA) is an aggressive type of bile duct cancer characterized by a high mortality rate due to difficulty in early diagnosis and ineffective treatment. The pathogenesis and progression of CCA have not fully understood. Studies suggest that overexpression of Notch genes and their response elements (NREs) promote development and progression of CCA. Thus it could be a novel approach in revealing mechanism, diagnosis and treatment of CAA to regulation of Notch signaling pathway. It is known tha non-coding RNAs (ncRNAs) are RNA molecules that do not encode proteins. However, emerging evidence suggest that a number of ncRNAs can indeed encode peptides that play roles in normal cell functions or human diseases including cancers. The proposed study is aimed to demonstrate that lncRNA-encoded micropeptides can play a role in Notch signaling pathway in cholangiocarcinoma. To reach the objective, following approaches will be used. 1) generating a CRISPR single guide RNA (sgRNA). Up to date, none of findings is ready for report.				
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**1. INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The proposed study is aimed to demonstrate that lncRNA-encoded micropeptides can play a role in Notch signaling pathway in cholangiocarcinoma. To reach the objective, following approaches will be used. 1) generating a CRISPR single guide RNA (sgRNA) library against small open reading frames (sORFs) of selected lncRNA; 2) generating Notch response element (NRE) reporter cell line; 3) performing library screening through infection of SNU-1079 (one of CCA cell lines) carrying the reporter with this CRISPR sgRNA library; 4) recovering gRNA sequences through PCR amplification, deep sequencing for PCR products and identify enriched gRNA sequences by bioinformatics analysis; and finally 5) functionally characterizing genes corresponding to the gRNA clones by manipulating their expression through KO/rescue or ectopic expression; and determining whether the peptides in SNU-1079 cells play roles in regulating expression of Notch genes, genes mediated by Notch, and products from these genes by RTPCR, western blot and immunoassays.

**2. KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Cholangiocarcinoma (CCA), Notch genes and their response elements (NREs), non-coding RNAs (ncRNAs), a CRISPR single guide RNA (sgRNA).

**3. ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

**What were the major goals of the project?**

*List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.*

The proposed study is aimed to demonstrate that lncRNA-encoded micropeptides can play a role in signaling pathway in cholangiocarcinoma. To reach the objective, five (5) major tasks were set to accomplish:

Major Task 1: To generate a CRISPR single guide RNA (sgRNA) library against small open reading frames (sORFs).

Major Task 2: To generate Notch response element (NRE) reporter cell line

Major Task 3: To perform library screening.

Major Task 4: To recover gRNA sequences

Major Task 5: To functionally characterize gRNA candidates and to determine whether these regulatory genes are dysregulated in SNU-1079 cells

### What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

#### Major Task 1 To generate a CRISPR single guide RNA (sgRNA) library against small open reading frames (sORFs) (Completed)

We designed 5 sgRNAs against each for a total 800 targeted sORF. These sgRNAs were synthesized as a pool of single stranded oligonucleotide from IDT. As shown in Fig.1, sgRNA LINC01410-1 was one of examples and common adaptor sequences were shown in blue. After conversion of single stranded oligos into double stranded oligos by PCR, they were cloned into lentiCRISPR v2 (LCV2) (Fig. 1). The ligated products were transformed into E. coli cells by electroporation. Next day, E. coli transformants were directly harvested for plasmid isolation. At this stage, the lentiviral sgRNA library is ready for next stage of study, i.e., screening.

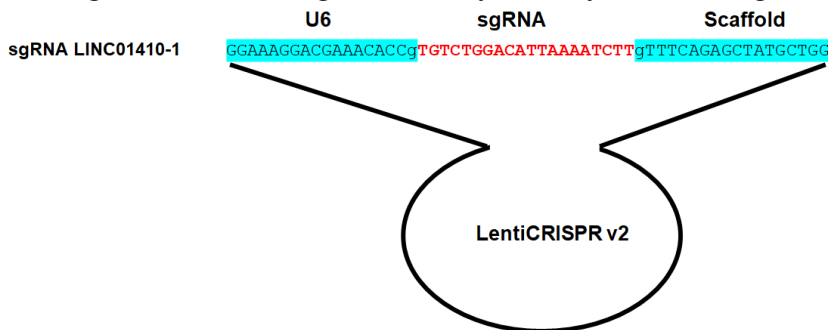


Fig. 1 Construction of sgRNA library against sORFs in LentiCRISPR v2.

Major Task 2: To generate Notch response element (NRE) reporter cell line (ready to initiate).

Major Task 3: To perform library screening (ready to start on January 2024 with newly recruited scientist).

Major Task 4: To recover gRNA sequences (ready to start on January 2024 with newly recruited scientist).

Major Task 5: To functionally characterize gRNA candidates and to determine whether these regulatory genes are dysregulated in SNU-1079 cells (ready to start on January 2024 with newly recruited scientist).

**What opportunities for training and professional development has the project provided?**

*If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. “Training” activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. “Professional development” activities result in increased knowledge or skill in one’s area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.*

Nothing to Report

**How were the results disseminated to communities of interest?**

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.*

Nothing to report

**What do you plan to do during the next reporting period to accomplish the goals?**

*If this is the final report, state “Nothing to Report.”*

*Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

Lab personnel has been recruited. It is expected to accomplish all tasks by the end of the next reporting period.

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

**What was the impact on the development of the principal discipline(s) of the project?**

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).*

Nothing to report.

**What was the impact on other disciplines?**

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.*

Nothing to report.

**What was the impact on technology transfer?**

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:*

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

**What was the impact on society beyond science and technology?**

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:*

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

**5. CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to report.

*Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.*

**Actual or anticipated problems or delays and actions or plans to resolve them**

*Describe problems or delays encountered during the reporting period and actions or plans to resolve them.*

Due to pandemic, it is hard to recruit lab personnel. Now the recruitment is going on. It is expected one scientist will be in the position by the end of year 2023.

**Changes that had a significant impact on expenditures**

*Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.*

No change.

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

*Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.*

No changes.

**Significant changes in use or care of vertebrate animals**

No changes.

**Significant changes in use of biohazards and/or select agents**

No changes.

**6. PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”*

Nothing to report.

- **Publications, conference papers, and presentations**  
*Report only the major publication(s) resulting from the work under this award.*

**Journal publications.** *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume; year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

**Books or other non-periodical, one-time publications.** *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

**Other publications, conference papers and presentations.** *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (\*) if presentation produced a manuscript.*

Nothing to report.

- **Website(s) or other Internet site(s)**

*List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.*

Nothing to report.

- **Technologies or techniques**

*Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.*

Nothing to report.

- **Inventions, patent applications, and/or licenses**

*Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.*

Nothing to report.

- **Other Products**

*Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:*

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report.

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

### What individuals have worked on the project?

*Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change”.*

#### Example:

Name: Mary Smith  
Project Role: Graduate Student  
Researcher Identifier (e.g. ORCID ID): 1234567  
Nearest person month worked: 5

No change.

### Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.*

Nothing to report.

### What other organizations were involved as partners?

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.*

*Provide the following information for each partnership:*

*Organization Name:*

*Location of Organization: (if foreign location list country)*

*Partner's contribution to the project (identify one or more)*

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report.
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## **8. SPECIAL REPORTING REQUIREMENTS**

**COLLABORATIVE AWARDS:** *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ers.amedd.army.mil> for each unique award.*

**QUAD CHARTS:** *If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments.*

- 9. APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*