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TITLE: Stathmin Phosphorylation as a Target for Blocking Metastasis in Prostate Cancer

PRINCIPAL INVESTIGATOR: Susan Kasper, PhD

CONTRACTING ORGANIZATION: University of Cincinnati

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14. ABSTRACT

Metastasis is a primary cause of cancer-related deaths, yet this process remains poorly understood. Stathmin (Stmn1) is an oncoprotein over-expressed in many cancers, including prostate cancer (PCa). While increased Stmn1 correlates with disease progression and poor prognostic outcome, however its role in metastasis is still being elucidated. Stmn1 activity is controlled by four serines (S16, S25, S38, and S63) which are differential phosphorylation by 4 different pathways. Therefore, the purpose of this study is to determine which one of these serines (and associated pathway) regulates proliferation and which promotes metastasis. The **hypothesis** is that *the first serine, S16, is the predominant serine that regulates PCa cell proliferation and acts as a gatekeeper to inhibit a cascade leading to metastatic PCa*. To address this hypothesis, *Specific Aim 1* will determine the function of Stmn1 S16 and the inter-relationship between S16, S25, S38 and/or S63 phosphorylation in regulating cell proliferation and a malignant phenotype, *Specific Aim 2* will determine the impact of Stmn1 phosphorylation on metastasis using a zebrafish xenograft model and *Specific Aim 3* will determine the clinical relevance of the different phospho-Stmn1s by analyzing human Tissue Microarrays representing the range of prostate cancer progression from benign to metastatic cancer. This approach will identify the major Stmn1 phospho-forms expressed during the different stages of prostate cancer progression and determine whether a specific isoform could serve as a biomarker for prostate cancer progression.

15. SUBJECT TERMS NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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TABLE OF CONTENTS

1. INTRODUCTION: *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Subject: Metastasis is a primary cause of cancer-related deaths, yet this process remains poorly understood. Stathmin (Stmn1) is an oncoprotein over-expressed in many cancers, including prostate cancer (PCa). While increased Stmn1 expression correlates with disease progression and poor prognostic outcome, it is not known whether Stmn1 overexpression correlates with biological activity. **Purpose**: Our previous work demonstrated that eliminating Stmn1 protein expression only modestly decreased PCa cell proliferation; instead, loss of Stmn1 protein greatly induced metastasis. Therefore, it is essential to determine how Stathmin activity can be selectively manipulated to block PCa cell growth without increasing the risk of more aggressive metastasis. This knowledge is critical for the development of targeted new therapies that block tumor progression and kill tumor cells. Since Stmn1 activity is controlled by four serine residues (S16, S25, S38, and S63) which are differentially phosphorylated by 4 different pathways, the purpose of this study is to determine which one of these serines (and associated pathway) regulates proliferation and which promotes metastasis. **Scope**: Our **hypothesis** is that *the first serine, S16, is the predominant serine that regulates PCa cell proliferation and acts as a gatekeeper to inhibit a cascade leading to metastatic PCa*. To address this hypothesis, *Specific Aim 1* will determine the function of Stmn1 S16 and the inter-relationship between S16, S25, S38 and/or S63 phosphorylation in regulating cell proliferation and a malignant phenotype; *Specific Aim 2* will determine the impact of Stmn1 phosphorylation on metastasis using a zebrafish xenograft model *in vivo* to track tumor formation, cell migration and metastasis; and *Specific Aim 3* will determine the clinical relevance of Stmn1 phosphorylation in human prostate cancer progression using commercial antibodies to the 4 phosphorylated serines in Stmn1 to analyze human Tissue Microarrays representing the range of prostate cancer progression - from benign to metastatic cancer. This approach will identify the major Stmn1 phospho-forms expressed during the different stages of prostate cancer progression and determine whether a specific isoform could serve as a biomarker for prostate cancer progression.

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

Stathmin, Stmn1, Phosphorylation, Ca2+/calmodulin-dependent kinase II, CaMKII, metastasis, prostate, epithelial mesenchymal transition, EMT, human prostate TMA

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 major goals of the project are:

Specific Aim 1: Elucidate the mechanisms by which Stmn1 phosphorylation regulates PCa cell growth and metastatic potential.	**Months**
Major Task 1: Generate Stmn1 phospho-mutant CRISPR/Cas9 constructs and cell lines	
Subtask 1: Generate the phospho-Stmn1 constructs using CRISR/Cas9 system Cell lines used: none.	1-5

Subtask 2: Generate cell lines using phospho-Stmn1 CRISR/Cas9 constructs Cell lines used: DU-145 [ATCC]	3-12
Milestone(s) Achieved: Production of stable cell lines expressing Stmn1serine substitutions.	12
Major Task 2: Characterization of Stmn1 phospho-mutants in cell lines	
Subtask 1: Analysis of Stmn1 phospho-mutants using cell culture assays Cell lines used: DU-145 [ATCC] and derivative DU-145/Stmn1 phospho-mutants + DU-145/shStmn1[made in our lab]	3-24
Milestone(s) Achieved: Evaluation of Stmn1 phospho-mutants	24
Specific Aim 2: Determine the impact of Stmn1 phosphorylation on metastasis in a zebrafish xenograft model in vivo	
Major Task 1: Analysis of Stmn1 phosphorylation on tumor formation and metastasis in vivo.	
Subtask 1: Submit documents for ACURO approvals	1-4
Milestone(s) Achieved: Obtain ACURO approval	4
Specific Aim 3: Determine the clinical relevance of Stmn1 phosphorylation in human prostate cancer progression.	
Major Task 1: Characterization of Stmn1 phosphorylation in human PCa TMAs	
Subtask 1: Submit documents for ACURO approvals	1-4
Milestone(s) Achieved: Obtain ACURO approval	4
Subtask 2: Preparation and analysis of human PCa TMAs	4-15

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 shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Specific Aim 1: Elucidate the mechanisms by which STMN1 phosphorylation regulates PCa cell growth and metastatic potential.

Major Task 1: Generate the STMN1 phospho-mutant CRISPR/Cas9 constructs and cell lines

Subtask 1: Generate the phospho-STMN1 CRISR/Cas9 constructs

Multiple attempts of the CRISPR protocol didn't work. We recently learned that the CRISPR/Cas9 protocol for generating substitution mutations was not yet fully developed when we wrote the proposal, and that the protocol has now been adjusted and fully established. In the interim, we used the standard site directed mutagenesis strategy and successfully completed the project.

Subtask 2: Generate the cell lines stably expressing the STMN1 phospho-mutant constructs

Cell lines used: DU-145 [ATCC]

Developing 14 stably transfected lines was not possible within a 3 year timeframe. However, the STMN1 phospho-mutant constructs worked very well in transfection assays, and this approach was used to investigate the role of STMN1 phosphorylation in cell proliferation, migration and invasion. Two documents are provided:

(1) a preliminary draft of a manuscript studying the role of STMN1 S16 in cell cycle progression and metastatic potential (Paul Deford, for a PhD thesis), and

(2) a PhD thesis (Alison Pecquet) investigating the role of STMN1 S25 phosphorylation in promoting metastatic potential (all chapters, but excluding Chapter 6 which was on an unrelated project).

Major Task 2: Determine which STMN1 phospho-serine(s) regulates proliferation and/or metastatic phenotype

Subtask 1: Cell culture experiments to analyze cell proliferation, migration and invasion

This task has been nearly completed with only a few experiments left to do to confirm the results. Two documents are provided and any remaining experiments are indicated therein:

(1) a preliminary draft of a manuscript studying the role of STMN1 S16 in cell cycle progression and metastatic potential (Paul Deford, for a PhD thesis), and

(2) a PhD thesis (Alison Pecquet) investigating the role of STMN1 S25 phosphorylation in promoting metastatic potential (all chapters, but excluding Chapter 6 which was on an unrelated project).

Major Task 3: RNA-seq analysis to develop profiles that distinguish between cell proliferation and metastatic potential for prediction of PCa progression and/or metastasis

Subtask 1: RNA-seq, pathway analysis, and biological validation of key genes. The later date of this task is to ensure that the CRISPR/Cas9 cell lines are well-characterized and optimal cell lines are selected for the RNA-seq analysis.

Cell lines used: DU-145/STMN1 phospho-mutant, DU-145 and DU-145/shSTMN1cell lines

Multiple attempts of the Invitrogen CRISPR protocol didn't work. Therefore, we were unable to perform the RNA-seq analysis in this study. We recently learned that the CRISPR/Cas9 protocol for generating substitution mutations was not yet fully developed and that this contributed to the unsuccessful attempts in generating the substitution mutations for this study.

Major Task 4: Analysis of small molecule inhibitors with/without androgen deprivation to inhibit PCa cell growth

Subtask 1: Test small molecule inhibitors to individual phospho-serines with/without androgen deprivation in cell culture

Cell lines used: DU-145, DU-145/AR (made in our laboratory), LNCaP [ATCC], LAPC4 [provided by Dr. Charles Sawyers]

Given the depth of analysis, we focused on small molecule inhibitors that regulated STMN1 S16 and S25

phosphorylation. This task has been nearly completed with only a few experiments left to do to confirm the results. Two documents are provided and any remaining experiments are indicated therein:

(1) a preliminary draft of a manuscript studying the role of STMN1 S16 in cell cycle progression and metastatic potential (Paul Deford, for a PhD thesis), and

(2) a PhD thesis (Alison Pecquet) investigating the role of STMN1 S25 phosphorylation in promoting metastatic potential (all chapters, but excluding Chapter 6 which was on an unrelated project).

Milestone(s) Achieved:

1. We have completed the proposed tasks.
2. This work has provided training and dissertations for 2 PhD students and 2 manuscripts are currently in preparation.

Specific Aim 2: Determine the impact of STMN1 phosphorylation on metastasis in a zebrafish xenograft model in vivo

Major Task 1: Delineate the actions of STMN1 phosphorylation in mediating tumor formation and metastasis in vivo.

Subtask 1: Culture and provide cell lines, determine optimal drug doses, and perform xenograft experiments in vivo.

Cell lines used: DU-145 and DU-145/shSTMN1

Xenograft experiment without/with activation of S25 phosphorylation were performed and documented and discussed in Alison Pecquet's PhD thesis investigating the role of STMN1 S25 phosphorylation in promoting metastatic potential (please see Appendix).

Major Task 2: Determine the actions of PCa cells carrying STMN1 S/E and S/A substitutions in mediating tumor formation and metastasis

Subtask 1: Culture and provide STMN1 phospho-mutant cell lines, test mutations for their activity to promote/inhibit tumor formation and metastasis in vivo

Cell lines used: DU-145/STMN1 substitution mutations, DU-145 and DU-145/shSTMN1cell lines

The CRISPR protocol for generating substitution mutations was not yet fully developed when we wrote the proposal, and therefore we were not able to generate the STMN1 S/E and S/A substitution mutation cell lines for the *in vivo* zebrafish studies. We used the standard site directed mutagenesis strategy and successfully completed all the proposed cell-culture-based experiments. However, isolating sufficient transfected cells to perform the *in vivo* zebrafish studies would have required a significant amount of time and resources beyond that provided for the study.

Major Task 3: Pre-clinical analysis of small molecule inhibitors with/without antiandrogen treatment to inhibit tumor formation and metastasis in vivo

Subtask 1: Small molecule inhibitors to individual phospho-serines will be tested with/without androgen deprivation.

Cell lines used: DU-145, DU-145/AR

Given the depth of analysis, we focused on small molecule inhibitors that regulated STMN1 S16 and S25 phosphorylation. This task has been written up in considerable detail in the draft manuscript and PhD thesis provided.

Milestone(s) Achieved:

1. The casper/flk:mCherry zebrafish line was established and used to determine that HGF promoted metastasis of DU-145 tumor cells in Zebrafish *in vivo*.

Specific Aim 3: Determine the clinical relevance of STMN1 phosphorylation in human prostate cancer progression.

Major Task 1: human PCa TMA analysis

Subtask 1: Preparing TMAs (required when using antibodies to phosphorylated proteins), probing TMAs with phospho-serine specific antibodies

The TMAs for total STMN1 protein expression and phosphorylated serines S16, S25, S38, and S63 have been completed.

Subtask 2: Scoring of TMAs and analysis of the data

This task has been delayed because of the pandemic. We are now in the process of scoring the results. Since the TMAs represent the range of prostate cancer progression - from benign to metastatic cancer – this analysis will determine the major phospho-form(s) expressed during the different stages of prostate cancer progression. In addition, since these specimens have been molecularly characterized with clinical and pathological information available, including paired benign and tumor comparison, Gleason score, proliferation score, PSA levels, time to recurrence, heterogeneity within various metastatic sites in an individual, osseous versus soft lesions, and the most recent abiraterone/enzalutamide treatment, this data will allow an in-depth analysis of the correlation of Stmn1 phosphorylation with these variables.

Milestone(s) Achieved:

1. Processing of the human five PCa TMAs is complete.
2. Evaluation of the primary phospho-serines expressed in benign, low-grade, high grade, and metastatic PCa and correlation of STMN1 phosphorylation with disease progression, metastasis and antiandrogen treatment is in process.
3. A manuscript is in preparation.

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.

Paul Deford and Alison Pecquet are two PhD students working on this project. The PI has provided group and one-on-one training on all aspects of this project. There are group lab meetings every Monday morning to discuss topics of common interest, e.g., cell biology, molecular biology, normal prostate development, mechanisms of metastasis, lab protocols, and new technologies. We also hold one-on-one meetings every Monday afternoon to discuss the previous week's work in detail, to analyze and interpret data, and to plan the week's research activities. The students also attend several seminar series, including the Wednesday Department of Environmental Health seminar series and the Cancer Cell Biology seminar series. Other training activities included presenting their project at laboratory meetings, at the Seminar Series for students in the Division of Genetics and Molecular Toxicology, at the ImmunoTox seminar series, and as posters at the annual UC Graduate Student Research Forum.

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.

Paul Deford presented at the Immunotoxicology Seminar on Oct. 28th, 2019 in Cincinnati with a talk entitled: "*CAMKII-mediated Phosphorylation of Stathmin in the Promotion of Prostate Cancer*"

Both students presented an update of their research progress at their yearly Environmental Genetics and Molecular Toxicology seminar in the Spring of 2021:

Paul Deford presented on February 5th, 2021 with a talk entitled: "*CAMKII-mediated Phosphorylation of Stathmin in the Promotion of Prostate Cancer*".

Alison Pecquet presented on February 12th, 2021 with a talk entitled: "*Stathmin1 Phosphorylation as a Regulator of Metastatic Potential"*.

Paul Deford presented at the Immunotoxicology Seminar on March 30th, 2021 in Cincinnati with a talk entitled: "*HGF Regulation of Stathmin Serine 16 Phosphorylation Modulates Cell Cycle Progression"*.

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.

This is our final reporting period. However, we will continue to complete the work and are in the process of writing three manuscripts. Temporary titles are:

1. HGF/MET-mediated Phosphorylation of Stathmin Serine 16 Regulates Cell Cycle Progression But Not Metastatic Potential

2. Differential Stathmin Phosphorylation on Serine 25 Regulates Epithelial Mesenchyme Transition
3. Ontogeny of Stathmin Phosphorylation during Prostate Cancer Progression to Castration Resistance

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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).

1. We have developed a series of unique expression vectors that generate specific STMN1 phospho-mutant proteins that can be used in combination with other factors to investigate their role in multiple biological processes. These vectors will be made available upon publication to other researchers to further their work.
2. While HGF and MET are overexpressed in many cancers and MET/HGF signaling in cancer progression has been investigated, our study has discovered that MET/HGF selectively activates STMN1 phosphorylation on S16 and that S16 phosphorylation is required to drive cell cycle progression and tumor cell growth. Importantly, STMN1 S16 phosphorylation does not promote metastasis. Treatment with the selective MET inhibitor AMG337 (currently in clinical trial) inhibits these processes and induces cell death. Based on these observations, we would predict that a MET inhibitor in combination with ADT would increase the probably of killing PCa cells before they metastasize. Other studies have reported that AR signaling represses *MET* gene transcription and that in the treatment of CRPC, the suppression of androgens by ADT decreases AR activity and reverses this process to result in MET overexpression[1,2]. Given that *MET* is an oncogene that drives cancer progression, the combination of targeting MET and AR offers an attractive targeted therapeutic approach for the treatment of CRPC.
3. Our study has also discovered that HGF/MET differentially modulates STMN1 serine phosphorylation, where STMN1 S16 selectively promotes tumor cell growth while STMN1 S25 selectively drives tumor metastasis. Therefore phospho-STMN1 S16 and S25 could be used as biomarkers of tumor stage and/or progression. In addition, STMN1 S16 could be used as a target to develop drugs that selectively prevent STMN1 S16 phosphorylation and induce tumor cell death, thereby limiting the metastatic spread of PCa. Similarly, developing drugs that selectively prevent STMN1 S25 phosphorylation would target metastasis, however we would predict that of the two, targeting STMN1 S16 in combination with ADT would be more effective in decreasing tumor burden.
4. The TMA used in this study is one of the most complete TMAs developed to date, as it provides biopsy samples covering BPH and most stages of PCa as well as samples pre- and post-treatment and the development of CRPC. Thus, we anticipate that the TMA analysis together with the extensive amount of deidentified clinical data associated with these biopsies will provide in-depth information and new insights into the role of STMN1 phosphorylation from BPH to low grade PCa through to failure of treatment of castration resistant prostate cancer.

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.

Both HGF and MET are overexpressed in many cancers. Therefore, our findings using prostate cancer as a model will likely impact other cancers as well.

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*:

Changes in approach and reasons for change

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.

Nothing to Report.

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.

Nothing to Report.

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.

Nothing to Report.

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.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

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."*

- **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.*

As outlined above, three manuscripts are in process.

- **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 license**s

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*.

We have developed a series of unique expression vectors that generate specific STMN1 phospho-mutant proteins that can be used in combination with other factors to investigate their role in multiple biological processes. These vectors will be made available upon publication to other researchers to further their work.

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*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award).*

Name: Susan Kasper, PhD

No Change

Name: Paul Deford

No change

Name: Alison Pecquet

No change

Name: Saulius Sumanas, PhD

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.

The following R01 application was funded: NIH/NIDCR, R01 (PI: K.A.Burns) 07/01/19 - 06/30/24 "The Role of the Matrisome in Endometriosis Development" Goals: Determine the role of neutrophil and macrophage mediated signaling on uterine tissue attachment during the immune-dependent phase of endometriosis. My role: Co-Investigator Research effort: 12.75 % This does not impact the effort on the project that is the subject of this project 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.

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.*

Not applicable.

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.*

Attached are two documents:

(1) a preliminary draft of a manuscript studying the role of STMN1 S16 in cell cycle progression and metastatic potential (Paul Deford, for a PhD thesis), and

(2) a PhD thesis (Alison Pecquet) investigating the role of STMN1 S25 phosphorylation in promoting metastatic potential (all chapters, but excluding Chapter 6 which was on an unrelated project).

REFERENCES

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