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LC180462

TITLE: Identifying biomarkers of metastasis through biosynthetic tagging

PRINCIPAL INVESTIGATOR: Alexander Pertsemlidis, PhD

CONTRACTING ORGANIZATION: The University of Texas Health Science Center at San Antonio

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PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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The University of Texas Health Science Center at San Antonio
7703 Floyd Curl Drive
San Antonio, TX 78229-3900

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U.S. Army Medical Research and Development Command
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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Overall work was slowed by closure of labs, suppliers and service providers during the COVID-19 pandemic, and progress on Aim 2 was limited by a technical hurdle related to the mouse model of metastasis. We requested and were granted a no-cost extension to address both issues. We have made progress on the molecular biology proposed in Aim 1, including engineering metastatic and non-metastatic cell lines derived from a murine model of lung adenocarcinoma to stably express the enzymes needed for RNA biosynthetic labeling -- these will be used in wildtype mice. Unmodified cell lines were to be used in transgenic, RNA-labeling mice, but the cell lines were derived from 129Sv mice and don't behave exactly as expected in the transgenic C57BL/6 mice. With separate funding, we are establishing the required transgenic 129Sv mice by crossing the C57BL/6 transgenic mice onto the 129Sv background. We have amended our animal protocol to reflect use of the derived strain and have received local IACUC approval.

15. SUBJECT TERMS
Metastasis, miRNA, biosynthetic labeling

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
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TABLE OF CONTENTS

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

The current proposal is a novel combination of chemical and genetic tools to identify the tumor-specific miRNA secretome. We leverage a murine model of the extremes of lung cancer metastatic behavior, a protozoan enzyme that can selectively incorporate a modified base into RNA, and expression profiling by next-generation sequencing to identify miRNA markers of metastasis. Our hypothesis is that a recently-developed RNA tagging approach can be applied to isograft models based on cells that differ in their ability to metastasize, allowing us to determine whether specific miRNAs in serum and tissue are secreted by tumor cells or are expressed by the host, and to identify those that are associated with metastasis.

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

microRNA, RNA labeling, lung cancer, metastasis

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.

	Original Timeline	Proj/% Completion
Major Task 1: Derive and characterize cell lines that express thiol-labeled RNA	Months	Date
Subtask 1: Transfect UPRT plasmid into cell lines and screen for stable clones *Cell lines: 344SQ (metastatic) and 393P (non-metastatic) cell lines derived from the* $Kras^{LA1/+;}$ $p53^{R172H\Delta G}$ *murine model*		100%
Subtask 1a. Transfect UPRT plasmid into cell lines	1-3	11/19
Subtask 1b. Screen for stable clones	1-3	2/20
Subtask 1c. Quantify expression and select suitable clones	1-3	6/20
Subtask 2: Optimize conditions for optimal 4-TU incorporation in vitro		33%
Subtask 2a. Grow modified cell lines in 4-TU supplemented media	4-6	10/20
Subtask 2b. Biotin/streptavidin pull-down in cell lysate	4-6	10/20
Subtask 2c. Biotin/streptavidin pull-down in supernatant (miRNA)	4-6	10/20
Milestone(s) Achieved:		
344SQ-UPRT and 393P-UPRT cell lines		6/20
Optimized protocol for isolation of TU-tagged miRNAs		10/20
Major Task 2: Define the miRNA transferome	Months	
Subtask 3: Induce tumors based on UPRT+ cell lines in wt mice		0%
3a. Inject cell lines into mice with controls	7-8	2/21

3b. Sacrifice mice, harvest tumors, adjacent tissue and blood	7-8	4/21
3c. Extract miRNA from tumor, adjacent tissue and serum	7-8	4/21
Subtask 4: Induce tumors based on wt cell lines in tg UPRT+ mice		0%
4a. Inject cell lines into mice with controls	7-8	2/21
4b. Sacrifice mice, harvest tumors, adjacent tissue and blood	7-8	4/21
4c. Extract miRNA from tumor lysate, adjacent tissue and serum	7-8	4/21
Subtask 5: Profile expression of miRNAs		
5a. Fractionate pooled sera and tissue lysates by biotin pulldown	9	5/21
5b. Demonstrate sensitivity and specificity of host/isograft miRNA separation and quantitation	9	5/21
5c. Profile expression by next-generation sequencing	9-10	6/21
5d. Compare isograft serum profiles to host serum profiles to identify tumor-derived miRNAs that are markers of tumor presence	11-12	7/21
5e. Compare serum profiles from metastatic and non-metastatic models to identify serum miRNA markers of metastasis	11-12	7/21 7/21
5f. Evaluate candidate miRNA targets for association with metastasis and patient survival in public datasets	11-12	7/21
5g. Evaluate candidate transferred miRNAs as therapeutic targets	11-12	8/21
Milestone(s) Achieved:		
miRNA expression profiles of cell lines		0%
miRNA expression profiles of models: tumor, NAT and serum		0%
miRNA transferomes: tumor to/from NAT, tumor to serum		0%

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.

(1) Major activities

(Major Task 1) Derive and characterize cell lines that express thiol-labeled RNA. We have made progress on the molecular biology proposed in Aim 1, including engineering metastatic and non-metastatic cell lines derived from a murine model of lung adenocarcinoma to stably express the enzymes needed for RNA biosynthetic labeling. We designed and implemented two plasmids (based on D11 and D12, generously provided by Leo Bleris at UT Dallas). The first (shown in linear form in **Figure 1**) uses the D12 backbone to express spCas9 and mKate as a single ORF linked by a T2A self-cleaving peptide under the control of a CMV promoter and an sgRNA targeting the Rosa26 locus under the control of a U6 promoter. We included an NLS to ensure that the Cas9 protein localizes to the cell nucleus and a PEST domain to ensure that the mKate signal is rapidly degraded and therefore representative of spCas9 translation.

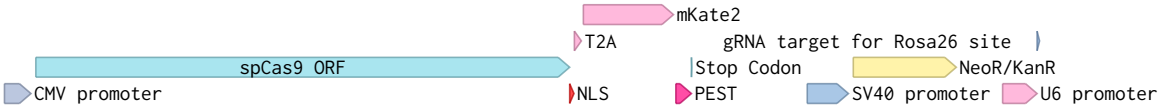

Figure 1. ZAP2-spCas9-mKate-Rosa26sgDNA (9480 bp).

The second plasmid (shown in linear form in **Figure 2**) uses the pcDNA3.1 backbone to provide a cassette for homology-directed repair of the Cas9-mediated cut of the Rosa26 locus, including flanking arms, CMV-driven expression of HA-tagged UPRT and mKate, and PGK-driven expression of puroR so that we can select for stable integration using puromycin and verify expression of UPRT by red fluorescence.

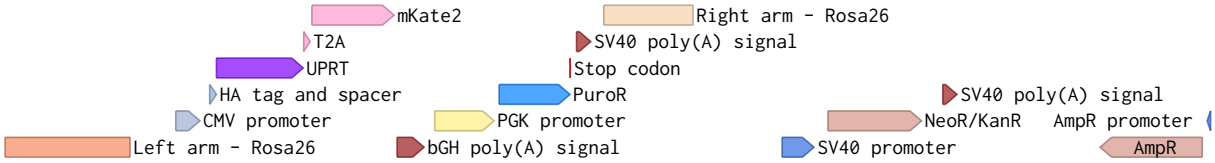

Figure 2. ZAP1-Rosa26L-HA-UPRT-mKate-puroR-Rosa26R.

These RNA-labeling cell lines will be used in wildtype mice.

(Major Task 2) Define the miRNA transferome. Unmodified cell lines were to be used in transgenic, RNA-labeling mice, but the cell lines were derived from 129Sv mice and don't behave exactly as expected in the transgenic C57BL/6 mice (see below). With separate funding, we are establishing the required transgenic 129Sv mice by crossing the C57BL/6 transgenic mice onto the 129Sv background. We have amended our animal protocol to reflect use of the derived strain, received local IACUC approval, obtained the needed strains, and started back-crossing.

(2) Specific objectives

(Subtask 1a, Subtask 1b) Transfect UPRT plasmids into cell lines and screen for stable clones. Transfection of 393P cells with the two constructs followed by puromycin selection yielded a single positive clone. Initial transfection and selection with 344SQ cells yielded no positive clones. A second round of transfections and selection yielded over 100 clones (**Figure 3**).

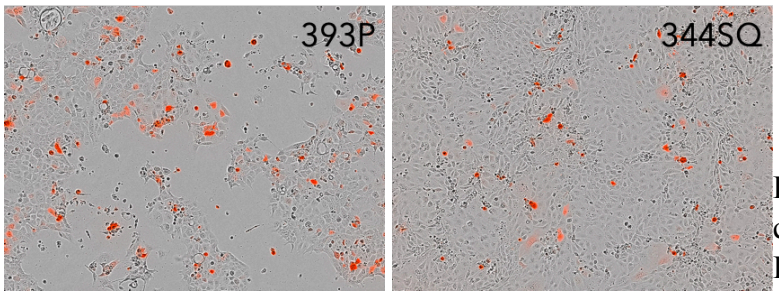

Figure 3. Live cell imaging of red channel fluorescence using an Essen IncuCyte FLR.

(Subtask 1c) Quantify expression and select suitable clones. We confirmed integration of the UPRT cassette into the Rosa26 locus using a specific PCR reaction overlapping the UPRT insert and the flanking Rosa26 region, and expression of UPRT by western blot (**Figure 4**).

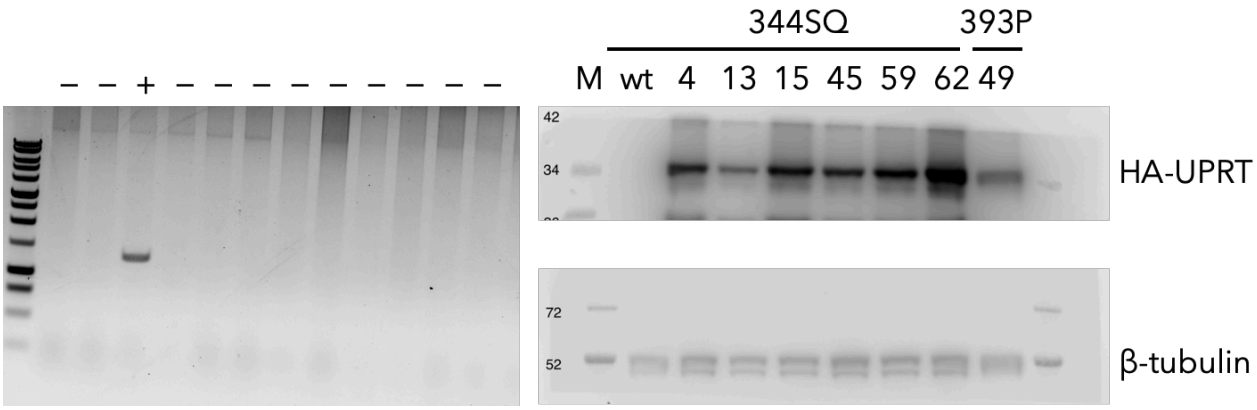

Figure 4. Confirmation of integration of UPRT into the Rosa26 locus.

(Subtask 2) Optimize conditions for optimal 4-TU incorporation in vitro. As shown in **Figure 5**, exposing UPRT-expressing 344SQ or 393P cells to 2.5-100 micromole concentrations of 4-thiouracil for 24 h had no effect on cell viability. Based on similar experiments with human cells, this was unexpected. Mouse cells may be more tolerant of incorporation of 4TUMP into RNA, or longer exposure times may be needed to see such effects.

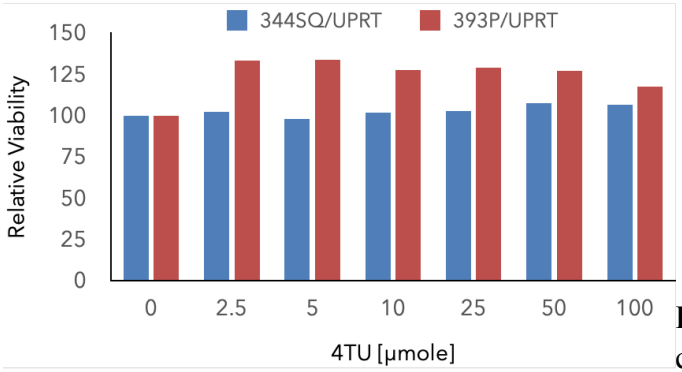

Figure 5. Assessing effects of 4TU/UPRT on cell viability.

(Subtask 4) Induce tumors based on wt cell lines in tg UPRT+ mice. Cryo-recovered B6;D2-Tg(Actb-Uprt)372Cdoe/J embryos were implanted into pseudopregnant females by the Jackson Laboratory; 19 pups were delivered. The first round of breeding yielded 36 mice, of which 69.4% carried the transgene. To date, we have bred 102 mice, of which nearly 70% carry the transgene, as shown in **Table 1**.

	Female	Male	Subtotal	%
WT	16	15	31	30.4
Tg	38	33	71	69.6

Table 1. Breeding of B6;TgUprt mice.

To confirm expression of Uprt, we took tail snips between days 10 and 21. Mice were manually restrained between the thumb and forefinger and the distal 2mm of the tail was excised with sterile scissors. No anesthesia or analgesia was used. The mice were monitored to assure hemostasis after being returned to their cages. DNA isolated from tail sips was assessed for Uprt expression by PCR, as shown in **Figure 6**.

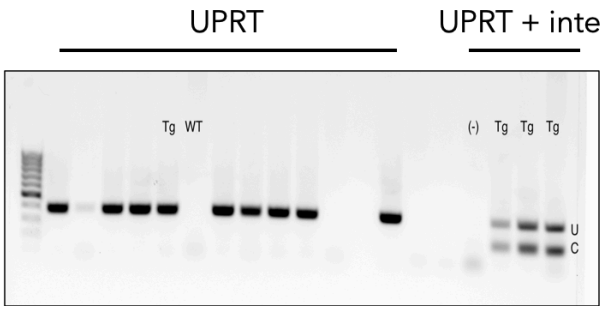

Figure 6. Genotyping of mice by PCR to confirm UPRT expression.

As noted above, we identified a discrepancy between the genetic background of our UPRT mouse (C57BL6) and our murine lung cancer cell lines (129Sv), which raises the issue of how tumor induction will differ between syngeneic and non-syngeneic backgrounds. To address the question of whether lung cancer cells derived from 129Sv mice induce tumors and metastasize in C57BL6 mice, we evaluated the ability of two mouse lung tumor cell lines (393P and 344SQ) to form lung tumor xenografts by injecting $1x10^6$ cells subcutaneously into 5 C57BL6 wild-type mice. Tumor growth was observed in two mice injected with metastatic 344SQ cells, but not in mice injected with non-metastatic 393P cells. All five mice were terminated when tumor size in 344SQ-injected mice reached 2000 cubic mm. We found that cells derived from 129Sv background mice xenografted into C57BL6 mice do not form tumors as expected (**Figure 7**).

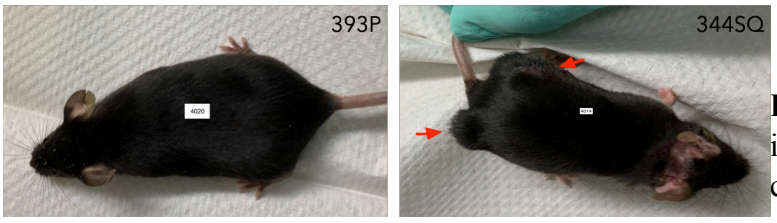

Figure 7. C57BL6/UPRT mice injected sub Q with 393P or 344SQ cells for 8 weeks.

As there are no 129Sv UPRT transgenic mice available commercially, we decided to back-cross our C57BL6 UPRT mice onto the 129Sv background to derive 129Sv;TgUprt. Generally, six back crosses are required to minimize C57BL6 background. We have generated 12 mice to date with 58% carrying the Uprt transgene.

	Female	Male	Subtotal	%
WT	1	4	5	41.7
Tg	6	1	7	58.3

Table 2. Breeding of 129Sv;TgUprt mice.

(3) Other achievements

Recognizing that we will need to monitor tumor growth in vivo, we established UPRT/luciferase-expressing cell lines (**Figure 8**).

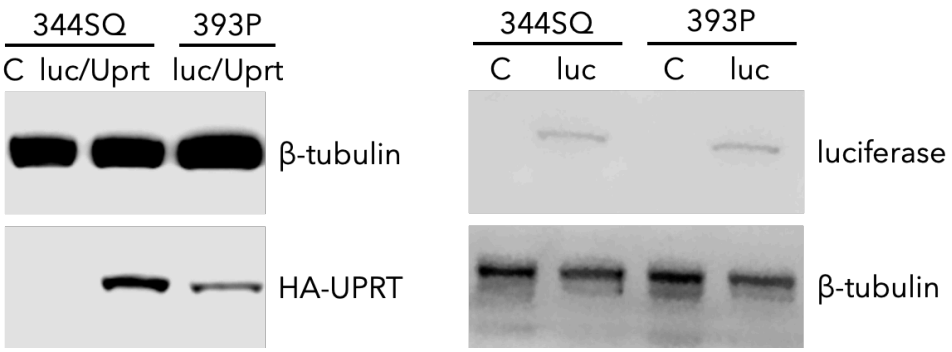

Figure 8. Confirmation of HA-UPRT and luc expression in 393P and 344SQ cell lines.

(4) Stated goals not met

None beyond what is described above.

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.

Training opportunities included greater exposure to bioinformatics methods through presentation and discussion of relevant techniques in our weekly group meetings. Topics covered to date include current-generation sequencing technologies, RNAseq data management and analysis, small RNA and large RNA mapping, and derivation of relative RNA expression.

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.

One we complete derivation of the UPRT transgenic 129Sv mouse, we will continue with the activities outlined in Major Task 2: "Define the miRNA transferome". We will induce tumors based on UPRT-expressing 393P (primary) and 344SQ (metastatic) cells in wt 129Sv mice, and unmodified 393P and 344SQ cells in UPRT-expressing 129Sv mice, harvest tumors, adjacent tissue and blood, isolate labeled miRNA, profile expression by NGS, compare profiles to identify differentially-expressed miRNAs that are associated with metastasis, and validate candidate miRNAs as therapeutic targets in vitro.

4. **IMPACT:** D*escribe 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:*

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.

Based on the closure of labs, suppliers and service providers during the COVID-19 pandemic, and the technical hurdle related to the mouse model of metastasis, we requested and were granted an extension of the project through 8/31/2021. Nothing additional 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.

As described above.

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.

The changes outlined above did not have a significant impact on overall expenditures, but did draw out the timeline on which award funds will be expended.

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

Not applicable.

Significant changes in use or care of vertebrate animals

We needed to expand our use of vertebrate animals to allow for additional breeding to move the UPRT transgene from the original C57BL6 background to the 129Sv background. This was approved by our IACUC on 5/18/20.

Significant changes in use of biohazards and/or select agents

None.

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.

  Nothing to Report.

  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.*

If transfer of the UPRT transgene to the 129Sv background is successful and the 129Sv-derived models of primary tumor formation and metastasis behave as expected, we will have contributed a new mouse model for the study of (murine) lung cancer metastasis.

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".

Name:	Alexander Pertsemlidis
Project Role:	PD/PI
Research Identifier:	0000-0003-1624-9372
Nearest person month worked:	1
Contribution to Project:	Dr. Pertsemlidis supervised the overall project, reviewed and analyzed data, interpreted results, and reported progress.
Funding Support:	NSF, CPRIT, William and Ella Owens Medical Research Foundation
Name:	Yiqiang Zhang
Project Role:	Research Scientist
Research Identifier:	0000-0003-0843-5394
Nearest person month worked:	6 (partially funded from other sources)
Contribution to Project:	Dr. Zhang performed all molecular biology, cell biology and animal work for the project. He designed and constructed expression cassettes, stably integrated them into cell lines, isolated and validated clones, and evaluated clone behavior in cell culture assays. He also maintained the mouse colony and derived new crosses.
Funding Support:	NSF, William and Ella Owens Medical Research Foundation
Name:	Shaimar Gonzalez
Project Role:	Graduate Student
Research Identifier:	
Nearest person month worked:	1
Contribution to Project:	Ms. Gonzalez participated in discussions of experimental design, data management and analysis, and interpretation of results. She also presented on bioinformatics methods relevant to different aspects of the project.
Funding Support:	NIDCR, Ford Foundation

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.

Active support of Drs. Pertsemlidis and Zhang changed during the reporting period with the close of the award from the William and Ella Owens Medical Research Foundation, the addition of pilot awards from the GCCRI and the Long School of Medicine, and the addition of a four-year award from the NSF.

These changes in active support do not impact the effort of Drs. Pertsemlidis and Zhang on the current project.

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.*

Organization Name: The University of Texas at Dallas
Location of Organization: 800 W Campbell Rd, Richardson, TX 75080
Partner's contribution to the project:
In-kind support
Collaboration

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.*