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TITLE: Functions of IL-1alpha and ESE-1 in Reactive Stroma Modulation of the Immune Landscape in Prostate Cancer

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14. ABSTRACT

The purpose of this project is to understand the role and mechanisms of IL-1alpha in regulating the ontogeny and differentiation of mesenchymal stem cells (MSCs) to immune reactive fibroblasts in prostate cancer. Aim 1 is to address ontogeny of immune reactive fibroblasts from perivascular MSCs. Aim 2 is to address the role of the ELF3 transcription factor. Aim 3 is to address how the recruitment and biology of immune reactive fibroblasts regulate the immune landscape in experimental tumors. Progress has been made in engineering prostate cancer cells for overexpression of IL-1alpha and in knocking down ELF3 expression in MSCs. We have generating three-dimensional organoids as proposed. We have also generated the NG2-cre/Esr1*/ R26-stop-EYFP and the NG2-cre/Esr1*/ R26-stop-EYFP / *Elf3* fl/fl mice, which have now been backcrossed (10 generations) into the C57BL/6 background. We have been successful in generating allograft tumors of TRAMP C1D cancer cells in the reporter mice. We were able to show that perivascular MSCs are the source of the IL-1alpha induced fibroblasts in vivo and that ELF3 mediates IL-1alpha action. Progress has been made in each Aim.

15. SUBJECT TERMS

Mesenchymal stem cells, prostate cancer, Il-1alpha, ELF3 transcription factor, reactive stroma

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ANNUAL RESEARCH REPORT (DAMD W81XWH-17-1-0605):

1. INTRODUCTION:

This project is focused on understanding the evolution of immune-regulatory carcinoma-associated fibroblasts (CAFs) in the tumor microenvironment of prostate cancer. CAFs are key regulators of the tumor microenvironment biology, including angiogenesis and immune surveillance. Understanding the co-evolution of CAFs during tumorigenesis and the key regulators of CAF biology is important to evaluating methods to target CAF biology as a way to target tumor progression. The project is focused on determining how IL-1alpha, expressed by carcinoma cells regulates CAF recruitment from perivascular mesenchymal stem cell (MSC) progenitors and the role of the ELF3 transcription factor in mediating IL-1alpha effects in MSCs. It is our hypothesis that elevated IL-1alpha expressed by prostate carcinoma cells activates perivascular MSCs to expand/differentiate to an immune modulatory CAF that is immunosuppressive and tumor-promoting. Moreover, we believe that the ELF3 transcription factor is the key mediator of MSC activation and differentiation to the immune modulatory CAF phenotype. The overall goal of the proposed project is to identify key mechanisms that could be exploited therapeutically. Progress in year 01 has led to several key findings that have addressed the hypothesis and led to new concepts as to the biology of recruited CAFs, as discussed in the present Progress Report.

2. KEYWORDS:

Prostate Cancer
Tumor Microenvironment
Carcinoma Associated Fibroblasts
Interleukin 1alpha
ELF3 Transcription Factor

3. ACCOMPLISHMENTS:

3a. What were the major goals of the project?

The major goals of the project align with the approved SOW as stated below.

Major Task 1: To develop and use novel 3D carcinoma cell / microvasculature organoids in vitro and in vivo organoid models to assess key mechanisms of IL-1alpha induced activation of perivascular stem cells.

Subtask 1: Generate engineered prostate cancer cell lines for elevated expression of IL-1alpha and for IPTG-regulated shRNA to IL-1alpha for knockdown and control (scrambled) engineered cells.

Subtask 2: Generate and analyze 3D organoids with engineered prostate cancer cell lines and microvascular fragments isolated from the NG2-cre/Esr1*/ R26-stop-EYFP mice.

Subtask 3: Engineer HPS-19I cells for overexpression of WFDC1/ps20 and use in 3D organoids with engineered LNCaP cells.

Subtask 4: Submit ACURO documents

Subtask 5: In vivo xenograft of engineered LNCaP cells with vessel fragments from the NG2-cre/Esr1*/ R26-stop-EYFP mice.

Subtask 6: Construct allografts of engineered TRAMP C1 cells in syngeneic NG2-cre/Esr1*/ R26-stop-EYFP mice.

Major Task 2: To test how genetic ablation of *ELF3/Elf3* in human and mouse mesenchymal stem cells affects activation, proliferation and differentiation to an immune regulatory carcinoma-associated fibroblast

Subtask1: To generate HPS-19I human mesenchymal stem cells for inducible shRNA knockdown of *ELF3*.

Subtask 2: To Co-culture and generate 3D organoids with engineered LNCaP cells and HPS-19I cells engineered for *ELF3* knockdown.

Subtask 3: To Co-culture and generate 3D organoids with engineered LNCaP cells with vessel fragments from the NG2-cre/Esr1*/ R26-stop-EYFP / *Elf-3* fl/fl mice.

Subtask 4: Generate Xenograft models of engineered LNCaP cells with HPS-19I cells with IPTG inducible shRNA knockdown of *ELF3*.

Subtask 5: Generate Xenograft models of engineered LNCaP cells with microvascular fragments from NG2-cre/Esr1*/ R26-stop-EYFP / *Elf3* fl/fl mice and NG2-cre/Esr1*/ R26-stop-EYFP mice as control.

Subtask 6: To generate allograft tumors of engineered TRAMP C1 cells in both the NG2-cre/Esr1*/ R26-stop-EYFP / *Elf3* fl/fl mice and control NG2-cre/Esr1*/ R26-stop-EYFP mice.

Major Task 3: To determine the role of IL-1alpha and ELF3 in regulating the immune landscape in experimental prostate cancer tumors and how this associates with rate of tumor growth.

Subtask 1: To generate allografts of TRAMP C1 cells in syngeneic mice.

3b. What was accomplished under these goals?

Major Task 1, Subtask 1: We have generated human LNCaP prostate cancer cells with engineered overexpression of IL-1alpha. We also have human PNT1A prostate epithelial cells engineered to overexpress IL-1alpha. Per the proposal, we have also generated mouse TRAMP C1D cells engineered to over express IL-1alpha. Hence, considerable progress has been made in

engineering prostate cells for overexpression of IL-1alpha. We have not yet generated cell lines with knockdown of IL-1alpha.

Major Task 1, Subtask 2: We have generated 3D organoids made with prostate cancer cells and mouse microvascular fragments in preliminary studies to date. We were able to culture these for 3 days and then inoculate them into syngeneic mice as discussed in Subtask 5 below. Initial experiments used microvascular fragments from Wfdc1 null mice as we had published evidence that low expression of Wfdc1 was associated elevated wound repair responses. We have not yet made these 3D organoids with microvascular fragments isolated from the NG2-cre/Esr1*/ R26-stop-EYFP mice.

Major Task 1, Subtask 3: We have not yet engineered HPS-19I cells for overexpression of WFDC1/ps20 and used them in 3D organoids with engineered LNCaP cells. We have experimented with Wfdc1 knockdown cells derived from microvascular fragments to conduct preliminary investigations related to Subtask 2 and Subtask 5. We were able to co-culture organoids of prostate cancer cells mixed with microvascular fragments derived from the Wfdc1 null mice. This is further discussed in Subtask 5 below. Given that we know that WFDC1/ps20 is expressed at a relatively high level in wild type HPS-19I we do not see as much of a need to overexpress this in order to complete the key objectives of Major Task 1.

Major Task 1, Subtask 4: We have submitted, modified and received ACURO permission for the proposed experiments. This subtask is completed.

Major Task 1, Subtask 5: We have not yet initiated in vivo xenograft studies using engineered LNCaP cells with vessel fragments from the NG2-cre/Esr1*/ R26-stop-EYFP mice. We have conducted the initial experiments to assess experimental conditions and feasibility of our co-culture and implantation protocols in order to optimize conditions for this Subtask. We were able to co-culture TRAMP C1D prostate cancer cells with microvascular fragments from the Wfdc1 null mice for three days and then implant them to produce in vivo xenografts. Remarkably we observed a large tumorigenesis with remarkable glandular histopathology in one mouse with good tumor-take.

Major Task 1, Subtask 6: We focused considerable effort on this Subtask in year 01. As proposed, we have generated allografts of engineered TRAMP C1D (engineered to overexpress IL-1alpha or vector control) in syngeneic NG2-cre/Esr1*/ R26-stop-EYFP mice. We made progress in optimizing tamoxifen dose scheduling relative to allograft initiation for lineage tracing experiments as proposed. Dosing tamoxifen on day 1, injection of engineered cells on day 2 with daily dosing of tamoxifen to day 5 gave reliable expression of EYFP in perivascular MSCs and their lineage recruited into the allografts. We have also optimized the timeline for these experiments. We evaluated allografts in mice at day 7, day 21 (3 weeks) and day 77 (11 weeks) post injection of either IL-1alpha engineered or vector control TRAMP C1D cells. We have observed recruitment of labeled CAFs derived from NG2-positive perivascular MSCs in vivo in both control and IL-1alpha overexpressing tumors at the day 7 and day 10 time points. At day 21 the IL-1alpha expressing tumors exhibit an increase in labeled CAFs but considerable heterogeneity was observed. In contrast, the day 77 (11 week) allografts exhibited dramatic differences. Labeled CAFs were observed throughout the tumors in the IL-1alpha expressing

allografts, whereas tumors were resorbed in the control allograft mice. IL-1alpha expressing allograft tumors exhibited a high degree in inflammatory infiltrate as evidenced by many leukocytes. EYFP+ CAFs were interspersed with TRAMP C1D prostate cancer cells in these allograft tumors. These studies provide proof of concept that IL-1alpha overexpression by prostate cancer cells results in recruitment of perivascular MSCs into the reactive stroma compartment. This is associated with an inflammatory infiltrate. We do not yet know the composition of the infiltrate and this is the subject of Major Task 3. Data from this subtask shows that TRAMP C1D cells are not capable of forming sustainable tumors under the conditions we have established without the overexpression of IL-1alpha. The lineage tracing study showed that is associated with recruitment of CAFs derived from microvascular pericyte MSCs that is consistent with our stated hypothesis. Accordingly, Subtask 6 is nearly complete. We are in the process of preparing these data for publication.

Major Task 2, Subtask 1**:** We have generated HPS-19I human mesenchymal stem cells with knockdown of *ELF3*. We have, to date, focused on siRNA knockdown for our preliminary experiments associated with this Subtask in order to better establish the role of ELF3 in MSC differentiation to an immune reactive CAFs. These studies have shown that ELF3 is the key mediator of IL-1alpha action induced differentiation of MSCs to CAFs per our hypothesis. We have discovered that inducible shRNA knockdown of gene expression to not be very reliable or easy to engineer with the HPS-19I cell line. This MSC cell line tends to differentiate to non - proliferative cells when using antibiotic selection media. Furthermore, we were able to show that knockdown of ELF3 resulted in restricted proliferation. Accordingly, as an alternative approach, we have used CRISPR-Cas9 system to knockout ELF3 in MSCs. This again resulted in a proliferation issue, hence we will be investigating other inducible ways to use CRISPR-Cas or shRNA without selection antibiotics. We have successfully used a fluorescent tag on engineered cells followed by Flow cytometry to sort out (select) positive cells with HPS-19 I MSC cells. We will likely use this method. Accordingly, we have not yet carefully evaluated this in the setting of co-culture with IL-1alpha expressing prostate cancer cells or with application of IL-1alpha to these cells. Importantly, however, we have demonstrated that knockdown of ELF3 resulted in a restriction of expression of cytokines and chemokines associated with the immune reactive CAF phenotype. These studies have now shown that the immune reactive fibroblast phenotype is dependent on ELF3 action in mediating IL-1alpha responses. These data are being prepared for publication.

Major Task 2, Subtasks 2-5: We have not yet initiated these experiments, as proposed, owing to new data gained during year 01 regarding MSC differentiation and the role of ELF3 in this process. In conducting Major Task 2 preliminary experiments, we found that IL-1alpha induced HPS-19I MSCs were terminally differentiated to what we characterize as immune reactive fibroblasts. Our previous work has shown that these MSCs and marrow-derived MSCs can be induced to prototypical myofibroblasts, also associated with the tumor microenvironment and that this is induced by TGF-beta and mediated by the RUNX1 transcription factor (Kim et al. 2014. *Proc Natl Acad Sci U S A*. 111(46):16389-94. doi: 10.1073/pnas.1407097111. PubMed PMID: 25313057**)**. Of direct impact to our Task 2 studies, we found that MSCs induced to immune reactive CAFs by IL-1alpha are subsequently restricted from TGF-beta induction to myofibroblasts. This is an important finding, since it suggests that the IL-1alpha induced, and ELF3 mediated immune reactive fibroblast is not a plastic change or phenotype, yet rather an

induced differentiation state that is irreversible. This finding has considerable relevance to our studies going forward. Furthermore, we found that activation of the Hippo pathway is likely responsible for this induced differentiation. These experiments found that phosphorylated YAP (that is sequestered in the cytoplasm) is associated with IL-1 alpha induced immune reactive fibroblasts only when they are co-stimulated with TGF-beta. We are now trying to determine if this is the key mechanism that restricts TGF-beta induction to the myofibroblast phenotype. This is critically important to understand in the project, since myofibroblasts are considered to be immune suppressive in the tumor microenvironment, whereas our data to date shows that IL-1alpha induced immune reactive fibroblasts promote immune infiltrate in this environment (as discussed in Major Task 1, Subtask 6 above) and are associated with elevated tumor growth (Major Task 1, Subtask 6). Although we do not know the composition of this infiltrate, these data suggest that pericyte MSCs can be driven to at least two differentiated phenotypes associated with emergent biology. One that is mediated by IL-1alpha/ELF3 and one mediated by TGF-beta/RUNX1 and that these pathways are mutually exclusive. This suggests, the reactive stroma tumor microenvironment can be polarized to either an immune surveillance state by immune reactive fibroblasts (Il-1/ELF3 mediated) or to an immune suppressive state by myofibroblasts (TGF-beta/RUNX1 mediated). Moreover, activation of the Hippo pathway (YAP activity) could be the switch that mediates which differentiated phenotype the MSCs will route. Accordingly, considerable progress has been made in understanding the sequellae of events and cell ontogeny in pericyte (MSC) induction to reactive stromal phenotypes and the key factors that mediate these sequences and the IL-1alpha/ELF3 induced sequence, and subsequent biology, which are the focus of this project. We are now much closer to further understanding of the process and regulators, which is the overall goal of this Major Task and the project as a whole. The finding of these Major Task 2 associated experiments will guide the rest of the experiments, particularly the Major Task 3 in vivo experiments.

Major Task 2, Subtask 6: We have focused considerable effort in year 01 to generate the crosses and backcross these mice into the C57BL/6 background required for the allograft experiments of this Subtask and Major Task 3. To date, we now have 10 generations of NG2-cre/Esr1*/ R26-stop-EYFP / *Elf3* fl+/- mice backcrossed into the BL6 background and are preparing to complete this Subtask and Major Task 3. These crosses are also essential for completion of Subtasks 3 and 5 of Major Task 2 studies.

Major Task 3, Subtask 1: There is only one subtask for this aim and we have made considerable progress in this Major Task. We have engineered TRAMP C1D prostate cancer cells for overexpression of IL-1alpha and vector control. We have also generated allografts in the control NG2-cre/Esr1*/ R26-stop-EYFP mice, as outlined in Major Task 1. Importantly, these control allograft tumors support our hypothesis that overexpression of IL-1alpha, but not basal expression, results in sustained tumorigenesis of the engineered TRAMP C1D prostate cancer cells in syngeneic NG2-cre/Esr1*/ R26-stop-EYFP mice. Experiments were delayed in using the NG2-cre/Esr1*/ R26-stop-EYFP / *Elf3* fl/fl mice owing to the time required for backcrossing into the C57BL/6 genetic background that is required for the TRAMP C1D allografts in this experimental setting (as outlined in Major Task 2, Subtask 6 above). In addition, we have made considerable progress in determining the optimal dosing schedule for tamoxifen to activate Cre recombinase expression in these mice.

3c. What opportunities for training and professional development has this project provided?

The project has been developed as a component of Ms. Linda Tran's Ph.D. Thesis project. She is supported 100% by this project as proposed in the approved Budget. Results have been discussed with her Thesis Advisory Committee. Ms. Tran's training program requires annual seminar presentation. Accordingly, we expect her to present this work at both the Dan L Duncan Comprehensive Cancer Center Symposia as a poster and future public seminars. Owing to her work and progress relative to this project, her Thesis Advisory Committee has given her formal permission to write her Thesis based on this work. Accordingly, she is now working on a manuscript that will present the progress reported in this Progress Report.

3d. How were results disseminated to communities of interest?

We are just completing year 01, therefore data has not been presented at a national meeting. Results have been discussed in intra-lab meetings. In addition, results have been discussed in Ms. Tran's Thesis Advisory Committee meetings as indicated above. We are planning to present a poster on these results at the Dan L Duncan Comprehensive Cancer Center annual symposia in March of 2019. Ms. Tran will also provide public seminars in the next year. Ms. Tran has presented this work, in part, during mini-seminar sessions presented as a component of her training program. As mentioned above, Ms. Tran is currently working on a draft of a manuscript that we hope to submit for publication in late 2018 or early 2019.

3e. What do you plan to do during the next reporting period to accomplish the goals?

Major Task 1: We plan to follow the proposed experimental plan and will focus on the recombination of vessel fragments derived from mice with reporter for NG2 positive pericyte MSCs and implantation of these organoids in vivo. As many of the in vitro studies have been addressed in our data to date, we believe focusing on the proposed in vivo studies will be the best use of time and resources during year 02. We plan to conduct some in vitro organoid experiments to gain a better understanding of the differentiation phenotype of NG2 positive MSCs, as proposed.

Major Task 2: We plan to follow the proposed experimental plan and will address how best to knockdown ELF3 given the limitations discovered in year 01. A major thrust will be to gain the most information we can using an siRNA knockdown as this does not growth arrest nor differentiate the MSCs. A major effort will be made to further elucidate the mechanisms and biology affected by ELF3 knockdown, the major goal of Task 2. We will continue to evaluate this in light of the new data regarding the role of the Hippo pathway in this process. It is now apparent that YAP activity in the nucleus and phosphorylated sequestration of YAP in the cytosol likely plays an important role in the mechanisms of IL-1alpha/ELF3 induced differentiation of MSCs to the immune reactive CAF phenotype. We plan to dissect these mechanism during year 02.

Major Task 3: We have made major progress in Task 3 experiments, as proposed. We plan to follow the rest of the proposed experiments to probe the immune landscape in these tumors. This will entail further dissection of the immune components of the tumor microenvironment in the experimental allograft mice. In addition, now that we have backcrossed the NG2-cre/Esr1*/ R26-stop-EYFP / *Elf3* fl/fl mice for 10 generations, we can begin to use these for the TRAMP C1D allografts that overexpress IL-1alpha and explore the in vivo role of ELF3 in the recruitment and/or differentiation of immune reactive CAFs and how this affects the immune microenvironment, as proposed.

4. IMPACT:

4a. What was the impact on the development of the principal discipline(s) of the project?

Data gained to date has changed the way we think about the co-evolution of immune reactive fibroblasts and myofibroblasts (which are likely immune suppressive) in the tumor microenvironment. Since both of these reactive stroma-associated cell types are derived from a common upstream MSCs (modeled by the HPS-19I cell line in our study), it is important to understand the ontogeny of these cells and the key pathways that regulate their differentiation. We have found that the ELF3 and the Hippo pathway (YAP/TAZ) are critical regulators in the induction of these classes of stromal cells in the emergent reactive stroma compartment in prostate cancer. This is important, as inhibitors of these pathways could be used to target the tumor microenvironment therapeutically.

4b. What was the impact on other disciplines?

The involvement of immune reactive fibroblasts and myofibroblasts is a hallmark of wound repair processes as well as tumor development. We believe that activation of pericyte MSCs to form reactive stroma (as induced by IL-1alpha/ELF3) is a general principle in many tissue disorders, including wound repair, diabetes tissue changes, and cardiovascular diseases. Accordingly, it is anticipated that the data gained will impact on the general field of wound repair, fibrosis, metabolic disorders, and tumorigenesis/tumor microenvironment biology of tumor systems other than prostate cancer. Accordingly, we feel this work will translate to understanding the mechanisms in other disorders.

4c. What was the impact on technology transfer?

Nothing yet to report.

4d. What was the impact on society beyond science and technology?

Nothing yet to report.

5. CHANGES / PROBLEMS

5a. Changes in approach and reasons for change:

There have been no major changes from the original plan, tasks, or procedures to conduct the research. We have found that knocking down IL-1alpha in our prostate cancer cell lines, as was originally proposed, is not likely necessary. Our data to date shows that wild-type prostate cancer cells have a basal expression of IL-1alpha that does not produce physiological results in our in vivo studies in Major Task 1 and 3 studies to date. We have shown that LNCaP cells have very low basal expression of IL-1alpha. Studies with overexpression of IL-1alpha (the key proposed experiments) showed a dramatic difference in phenotype as compared with vector control (wild type basal expression of IL-1alpha). Hence, there is no real need to knockdown levels of IL-1alpha gene expression (as a control), as they are apparently very low in basal conditions. We have also experienced somewhat of a delay in generating stable HPS-19I (MSC) cell lines with successful and inducible knockdown of ELF3, as proposed. Knockdown of ELF3 results in growth arrest and inability to continue the cell line in vitro. Hence, siRNA with more transient effects has generated key data to date and we believe we can complete the goal of the Major Tasks as proposed using siRNA knockdown and/or CRISPR-Cas procedures that would rely on flow cytometry to select cells. In addition, we do not feel it is important at the present time to overexpress WFDC1/ps20 in HPS-19I cells as outlined in Major Task 1, Subtask 3. Given that we know that WFDC1/ps20 is expressed at a relatively high level in wild type HPS-19I we do not see an obvious need to overexpress this in order to complete the key objectives of Major Task 1.

5b. Actual or anticipated problems or delays and actions or plans to resolve them:

We plan to address the cell engineering issues using other procedures as outlined in 5a. This will involve knockdown with siRNA with more transient effects has generated key data to date and we believe we can complete the goal of the Major Tasks as proposed using siRNA knockdown and/or CRISPR-Cas procedures that would rely on flow cytometry to select cells. We have also experienced some delay in the in vivo experiments owing to the need to backcross engineered mice into the C57BL/6 genetic background in order to use these for allografts. This has been resolved.

5c. Changes that had a significant impact on expenditures:

We have taken longer to generate stable knockdown of gene expression cell lines than anticipated during year 01. Moreover, we have had some delay in in vivo experiments owing to the need to generate the crosses and backcross these mice into the C57BL/6 background required for the allograft experiments of Major Task 2, Subtask 6 and Major Task 3 This has resulted in less expenditure of funds for year 01 than we expected. We anticipate using these funds in year 02.

5d. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:

We have had no changes in human cell line use, animals, biohazards or select agents.

5e. Significant changes in use or care of human subjects:

We have had no changes in use or care of human subjects.

5f. Significant changes in use or care of vertebrate animals:

We have had no changes in use or care of vertebrate animals.

5g. Significant changes in use of biohazards and/or select agents:

We have had no change in use of biohazards or select agents.

6. PRODUCTS:

6a. Publications, conference papers, and presentations:

The data generated from this project has not yet been published or presented at a national or local conference. This data has been shared in general laboratory meetings and in mini-seminars presented by Ms. Tran as a component of her training program. A manuscript is in preparation.

6b. Journal Publications:

The data generated from this project has not yet been published. A manuscript is in preparation.

6c. Books or other non-periodical, one-time publications:

Nothing to report.

6d. Other publications, conference papers, and presentations:

Nothing to report.

6e. Website(s) or other Internet site(s):

Nothing to report.

6f. Technologies or techniques:

We have developed a novel transgenic mouse with targeted and inducible expression of Cre recombinase by use of the NG2 promoter and this has been crossed with a mouse with floxed alleles of the Elf3 gene and backcrossed into the C57BL/6 genetic background (NG2-cre/Esr1*/ R26-stop-EYFP / *Elf3* fl/fl mice).

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6g. Inventions, patent applications, and/or licenses:

Nothing to report.

6f. Other Products:

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

7a. What individuals have worked on the project?

Name:	*David R. Rowley, Ph.D.*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	0000-0002-1297-8124
Nearest person month worked:	*3 person months*
Contribution to Project:	*Principal Investigator. Study design and supervision. Analysis of data. Writing all reports.*
Funding Support:	

Name:	*Linda Tran*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12 person months*
Contribution to Project:	*Ms. Tran conducted the experiments of each Task. She has also been involved in animal husbandry*
Funding Support:	

Name:	*Truong D. Dang*
Project Role:	*Research Assistant / Laboratory Technician*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*6 person months*
Contribution to Project:	*Mr. Dang provided project support by culturing all cells, ordering all supplies, management of the laboratory and assisting Ms. Tran with experiments.*
Funding Support:	

7b. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

There has been a change in active support for Dr. Rowley as follows:

Completed: Award RP140616. Cancer and Prevention & Research Institute of Texas (CPRIT) 08/01/2014 to 08/30/2017 (NCE to 2/30/18) (Rowley, D.R., PI) Title: Reactive Endosteum and Tenascin-C Biology in Metastatic Prostate Cancer. (Rowley: 25% Effort, 3 calendar months).

Awarded: The following grant has also been recently awarded to Dr. Rowley (PI) (we are presently awaiting the NGA): NIH (NCI) 1 R01 CA221946-01 Title: Osteogenic Niche Biology in Progression and Endocrine Resistance of Bone Metastases (Rowley: 15% Effort Proposed) The purpose of this application is to explore the role of estrogen receptor alpha in osteoblasts relative to bone metastases of both breast and prostate cancer. Two Specific Aims are proposed. Specific Aim 1. To address bone osteogenic niche - cancer interactions in differential steroid and anti-steroid action conditions using novel 3D osteogenic organoid approaches. This Aim will address the relative importance of ER alpha, ER beta, and AR in mediating estrogen, androgen, and anti-steroid actions in the genesis of a reactive osteogenic niche, how it affects breast and prostate cancer biology, and how it alters anti-steroid therapeutic efficacies. Specific Aim 2. To address mechanisms of anti-steroid (estrogen and androgen) biology in the osteogenic niche and how this affects colony initiation and progression of breast and prostate cancer in vivo. Using genetically engineered mouse models, novel tumor transplantation approaches and cutting-edge intravital microscopy, we will examine the impact of anti-steroid treatments on the osteogenic niche in vivo and how it leads to endocrine resistance. No Scientific or Budgetary overlap.

7c. What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

8a. Collaborative Awards:

Nothing to report

8b. Quad Charts:

Nothing to report

9. APPENDICES

Nothing to report