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CONTRACTING ORGANIZATION: University if Texas Southwestern Medical Center

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14. ABSTRACT

Osteolectin was recently identified as a protein secreted from bone mesenchymal stem cells (MSC) that promotes osteoblast differentiation when injected subcutaneously into mice. As NF1 patient fracture pseudarthroses are caused, at least in part, by defects in osteoblast differentiation of MSCs, the goal of this study is to evaluate whether osteolectin rescues osteoblast differentiation of patient pseudarthrosis-derived MSCs. Second as subcutaneously-injected Osteolectin improves skeletal development in mice, we are also testing whether osteolectin therapy may rescue skeletal development in an established mouse model of NF1 bone disease. If successful, this study may identify Osteolectin as a potentially novel therapy for NF1-associated skeletal disease.

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1. Introduction

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2. Keywords

Neurofibromatosis Type 1, pseudarthrosis, fracture, recombinant therapy, osteopenia

3. Accomplishments

What are the major goals of the project?

There are two primary goals of this study. The first primary goal of this study is to test whether recombinant Osteolectin protein rescues osteogenic differentiation of human patient-derived cell lines. The second goal of this study is to test whether skeletal development is rescued in an established conditional mouse model of Nf1 skeletal disease. Both goals are ~50% completed and we anticipate both will be completed during the approved extension period.

What was accomplished under these goals?

The first goal of this study is to test whether recombinant Osteolectin protein rescues osteogenic differentiation of human patient-derived cell lines. We are ~50% through completion of this Aim. Our results thus far show that the osteogenic potential of Osteolectin is greatly enhanced in the presence of the MEK inhibitor (MEKi) Trametinib. We originally proposed to utilize our previously-discovered expression biomarker to identify *NF1*$^{-/-}$ MSCs, as these MSCs express higher levels of the *EREG* gene. However because our combination therapy includes a MEKi, we are unable to utilize *EREG* as a stable expression biomarker. Therefore, we will utilize our time-series single-cell expression profiling approach (*manuscript in*

preparation) to test rescue of *NF1*-deficient patient fracture-derived MSCs. We anticipate completing this during the extension period.

The second goal of this study is to test whether skeletal development is rescued in an established conditional mouse model of Nf1 skeletal disease. Our results thus far show that, similar to resutls from our patient cells, that the combination of MEKi+Osteolectin enhances osteogenic differentiation of *Nf1*$^{+/-}$ mouse MSCs. Excitingly, our results also show that *Nf1*$^{-/-}$ MSCs, which fail to differentiate under standard osteogenic conditions, readily differentiate into osteoblasts with the combination therapy of MEKi+Osteolectin. We are currently breeding mice and purifying mouse Osteolectin protein to perform our *in vivo* experiment. We anticipate completing this goal during the extension period.

A total 85 mice have been utilized for this project thus far, which represents the progress made in establishing our animal colony for Specific Aim 2 (see Task #5).

Regarding activities outlined in the Statement of Work, we provide this updated table and a brief description of activies that are in progress:

Specific Aim 1(specified in proposal)	**Progress**
Single-cell Expression Assay	
1. HRPO approval	Completed
2. Design and validate *EREG*, *ALPL*, and housekeeping control Fluidigm Delta Gene single-cell qPCR assays	In progress
3. Assess recombinant CLEC11a to rescue osteogenic differentiation of NF1 pseudoarthrosis-derived human cell lines by single-cell qPCR	In progress
Milestone(s) Achieved	
Local IRB/IACUC Approval	Completed
Establish *CLEC11a*-expressing stable cell lines	Completed
Purify recombinant CLEC11a protein	In progress
Specific Aim 2	
Test Recombinant Clec11a in mice	
4. ACURO approval	Completed
5. Expand mouse colony for generating experimental animals	In progress
6. Confirm Xray/DEXA and micro-CT assessments in *Col2a1*-cre;*Nf1*$^{+/+}$ and *Col2a1*-cre;*Nf1*$^{fl/fl}$ mice.	In progress
7. Isolate and purify recombinant Clec11a protein	In progress
8. Assess skeletal response to recombinant Clec11a therapy in *Col2a1*-cre;*Nf1*$^{+/+}$, *Col2a1*-cre;*Nf1*$^{+/fl}$, and *Col2a1*-cre;*Nf1*$^{fl/fl}$ mice	In progress
Milestone(s) Achieved:	
Establish *Clec11a*-expressing stable cell lines	Completed
Initial IACUC protocol approval, including live-animal X-ray/DEXA analysis	Completed
Establish breeding pairs for colony maintenance	Completed

Task #2 and #3. We are in the process of installing our Fluidigm C1 instrument that will allow us to perform single-cell studies required to evaluate the impact of Osteolectin on differentiation of patient-derived cell lines. The preliminary results mentioned above utilized two patient-derived cell lines.

Task #5 and #6. We are in the process of expanding our breeding colony. Once experimental mice are available, we will complete tak #6. A total 85 mice have been used as part of this study thus far.

Task #7. We have isolated media from recombinant cell lines and are awaiting to purify the protein once restrictinos related to COVID-19 are released. We have isolated 2L of media which we anticipate will provide sufficient purified protein to complete Task #8.

Task #8. As mentioned, we are expanding our mouse colony to generate experimental animals and are in the process of purifying recombinant protein. Once these are completed, we will be positioned to complete Task #8.

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

Nothing to report

How were results disseminated to communities of interest?

Some of the results mentioned above were presented and discussed with patient representatives at the Children's Tumor Foundation annual meeting in 2019. Based on these discussions, we are now extending our expririments to investigate whether MEKi+Osteolectin may be a therapy for NF1-associated osteopenia.

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

Texas Scottish Rite Hospital for Children reently purchased a Fluidigm C1 instrument to perform time-series single-cell expression profiling of patient-derived cell lines. Once operational, we will perform the human cell-based experiments described above. Regarding the second goal, we have purified Osteolectin

protein and are in the process of concentrating and quantifying the purified protein. Concurrently, we are expanding our mouse colony to perform the *in vivo* experiment described above.

4. Impact

What was the impact on the development of the principal discipline of the project?

Results from this study have helped us to identify MEK-dependent and MEK-independent mechanisms of osteoblast differentiation. Through the course of this study, we hope to identify molecular singaling mechanisms that distinguish MEK-depdent and MEK-independent osteogenesis.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. Changes/Problems

Changes in approach and reasons for change?

Due to the need to utilize MEKi as a co-treatment with recombinant osteolectin protein (as described above), we have altered our approach to utilize our time-series single-cell expression profiling method to evaluate osteogenic differentiation of patient fracture-derived MSCs. Our alternative approach similarly utilizes the Fluidigm C1 platform.

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

We are currently within our approved extension period, and we anticipate all goals will be completed during this time. As described above, we are installing our new Fluidigm C1 instrument and plan to complete goal #1 as soon as it is operational. Furthermore, we are confirming our purification of mouse osteolectin protein and expanding our animal colony to have sufficient numbers of mice to evaluate the impact of MEKi+Osteolectin *in vivo*. We anticipate completing this study goal during the extension period.

Changes that had a significant impact on expendatures.

None.

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

None.

6. Products

Publications, conference papers, and presentations.

Some results from this study were presented at the annual Children's Tumor Foundation meeting in 2019.

7. Participants & Other Collaborating Organizations

Jonathan Rios
Role: Principal investigator
Researcher identifier:
Nearest person month worked: 1
Contribution to project: Dr. Rios is the principal investigator of the study and helps to direct the progress of the study. He meets weekly with Drs. Khalid and Paria.
Funding support: Texas Scottish Rite Hospital for Children

Aysha Khalid
Role: Post-doctoral fellow
Researcher identifier:
Nearest person month worked: 12
Contribution to project: Dr. Khalid is leading all animal model experiments for this study, including breeding mice, purifying protein, and performing ex-vivo differentiation experiments utilizing mouse MSCs.

Nandina Paria
Role: Research scientist
Researcher identifier:
Neraest person month worked: 3
Contribution to project: Dr. Paria is leading all experiments utilizing human patient-derived cell lines, including testing osteogenic differentiation.
Funding support: Texas Scottish Rite Hospital for Children

Has there been a change in the active other support of the PD/PI since the last reporting period?

The PI has received 1-year funding for an unrelated study. Dr. Rios is funded at 0.6 calendar months on this new grant.

What other organizations were involved as partners?

Nothing to report.

8. Special Reporting Requirements

Nothing to report.

9. Appendices